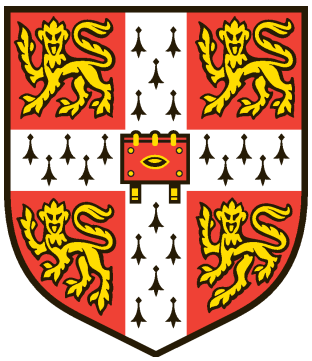
Using epidemiological principles and mathematical models to understand fungicide resistance evolution



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This dissertation is submitted for the degree of
Doctor of Philosophy

Declaration

This dissertation is the the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

This dissertation is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

This dissertation does not exceed the prescribed word limit of 60,000 words (excluding bibliography, figures and appendices) as specified by the Degree Committee of the Faculty of Biology.

James Elderfield

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Introduction

> *Fungi have been described as "a mutable and treacherous tribe", but that even this is something of an understatement is abundantly evident...*
>
> E. W. Buxton, *Heterokaryosis, saltation and adaptation*, 1960

1.1 What is a fungicide?

1.1.1 Definition and brief history

A fungicide is a substance that kills or inhibits the growth of fungi. We generally also include substances that target oomycetes in this definition, as although they are not fungi some aspects of their lifecycle are similar. Roughly 16 billion US dollars are spent worldwide on fungicides annually, and are used to reduce crop losses to disease which still stand at 20% (Jørgensen *et al.*, 2017). There are records of fungicide use as far as back as the middle of the 17th century, where brining and liming of grain was used to control bunt, even before the link between fungus and plant disease was identified (Morton & Staub, 2008). The use of Bordeaux mixture, copper sulphate and slaked lime, started in 1882 in France as a method of controlling downy mildew of grape and continues to this day, and is still widely used in organic farming (Morton & Staub, 2008). It was not until the mid 20th century that organic (in terms of chemistry) fungicides began to be widely used for plant disease control (Morton & Staub, 2008). Amongst the earliest organic fungicides were the dithiocarbamates and phthalimides; in general these newer classes of fungicide were more effective, less phytotoxic and easier to use (Morton & Staub, 2008). Modern fungicides are typically applied as foliar sprays or seed treatments.

1.1.2 Regulation

Fungicide use is now strongly regulated in most countries. The main motivation for these regulations is to limit the chance of harm to humans or the environment through misuse of chemicals (Gullino & Kuijpers, 1994). Consequently there is a lengthy and expensive approval process for new fungicides or even using existing fungicides for new applications. In recent years there have been updates to these regulatory standards in Europe and consequently a decrease in the number of fungicides available on the market, as existing

products have been withdrawn due to the high cost of re-registration or failure to meet new legislative requirements (Smith, 2014). In addition the cost of pesticide development has increased and the number of products in development has decreased (Phillips McDougall, 2013). With the reduction in the available products for disease control there is clearly therefore increased reliance on the effectiveness of the remaining fungicides.

1.1.3 Fungicide Resistance Action Committee (FRAC) Groups

Fungicides are split into groups defined by chemical structure and mode of action (MOA). MOA refers to which biochemical pathway the fungicide disrupts in the fungus. In addition to MOA, the target site is defined as the exact molecule (or molecules) that the fungicide interacts with to have its effect (Fungicide Resistance Action Committee, 2017a). A single fungicide may have only one target site, such as the succinase dehydrogenase inhibitors (SDHIs) which target a single enzyme involved in mitochondrial respiration (Avenot & Michailides, 2010). Alternatively a fungicide may have a range of MOAs or target sites, such as chlorothalonil that reduces glutathione (Tillman *et al.*, 1973), which is required for a range of biochemical pathways. Typically multi-site fungicides are effective against a broader range of organisms and thus can have unwanted off-target effects. The different target sites mean that fungicides can have a range of effects on a fungus, for example slowing mycelial growth or limiting spore production (Deliere *et al.*, 2010). It is typical to differentiate between protectant and eradicant activity; a given fungicide may show either of these activities to different degrees. The protectant activity of a fungicide is the control it provides when applied onto the plant before infection occurs, for example by inhibiting the germination of fungal spores. Eradicant activity is the capability of a fungicide to stop or slow an already initiated infection, and is often linked with the concept of a systemic fungicide which is absorbed into the plant and translocated throughout its tissues to a greater or lesser degree. One of the main reasons for the fungicide classification system is to aid in the management of fungicide resistance.

1.2 The problem of fungicide resistance

1.2.1 Definition

The Fungicide Resistance Action Committee defines fungicide resistance as (Fungicide Resistance Action Committee, 2017b),

> "An acquired, heritable reduction in sensitivity of a fungus to a specific anti-fungal agent (or fungicide)."

Resistance can vary from slightly reduced efficacy and the need to increase doses, such as prochlorax and fluquinconazole resistance in UK septoria during the 1990s (Mavroeidi

& Shaw, 2005), to complete inability to control the pathogen. The emergence of benzimidazole resistance in *Venturia inaequalis* (apple scab) in Germany and *Cercospora beticola* (Cercospora leaf spot) in Greece led to complete loss of activity after only two seasons of use (Staub, 1991). As well as impacts on food production, agricultural fungicide resistance can have implications for human health. *Aspergillus fumigatus* is a soilborne fungus that is pathogenic in humans and has likely developed resistance to medical DMI fungicides due to agricultural use of the same fungicides (Milgroom, 2015).

1.2.2 Mechanisms

In practice resistance typically manifests through genetic changes in the fungal population leading to one of a number of mechanisms: alteration of the target site, detoxification of the fungicide, overexpression of the target or exclusion of the fungicide (Dekker, 1986). Of these, alteration of the target site is by far the most common (Fungicide Resistance Action Committee, 2017a). As well as the obvious physiological and biochemical mechanisms, shifts in behaviour or phenology can also lead to a reduced effectiveness of applied fungicides (Birch & Shaw, 1997). The mechanism of resistance has implications for resistance monitoring, the evolutionary dynamics of the trait and its impact in an agronomic context.

1.2.3 Spread of resistance

Resistance can spread very quickly with the frequency of resistant isolates in a fungal population shown to be capable of increasing from around 5% to over 90% over the course of a single season under normal agronomic conditions (Fraaije *et al.*, 2002). As well as rapid increase at small spatial scales, the long range dispersal of fungal spores means that resistance can readily travel large distances over short timescales (figure 1.1). Due to the sample sizes which are required it is difficult to identify resistance below frequencies of around 0.1% (Milgroom, 2015), and detecting selection when the frequency of resistance is below 5% is impossible with current techniques (Walker *et al.*, 2017). Modelling work has suggested that resistance at these frequencies can rapidly increase to near-fixation over very few seasons (Hobbelen *et al.*, 2011a; Kable & Jeffery, 1980). Therefore currently undetected areas of low-level fungicide resistance may be able to rapidly increase to an economically-important degree. Furthermore laboratory studies may not be able to correctly identify the level of risk of resistance for novel fungicides, as was the case with phenylamides which were considered low risk but quickly developed extensive resistance problems (Leonard & Fry, 1989; Staub *et al.*, 1979).

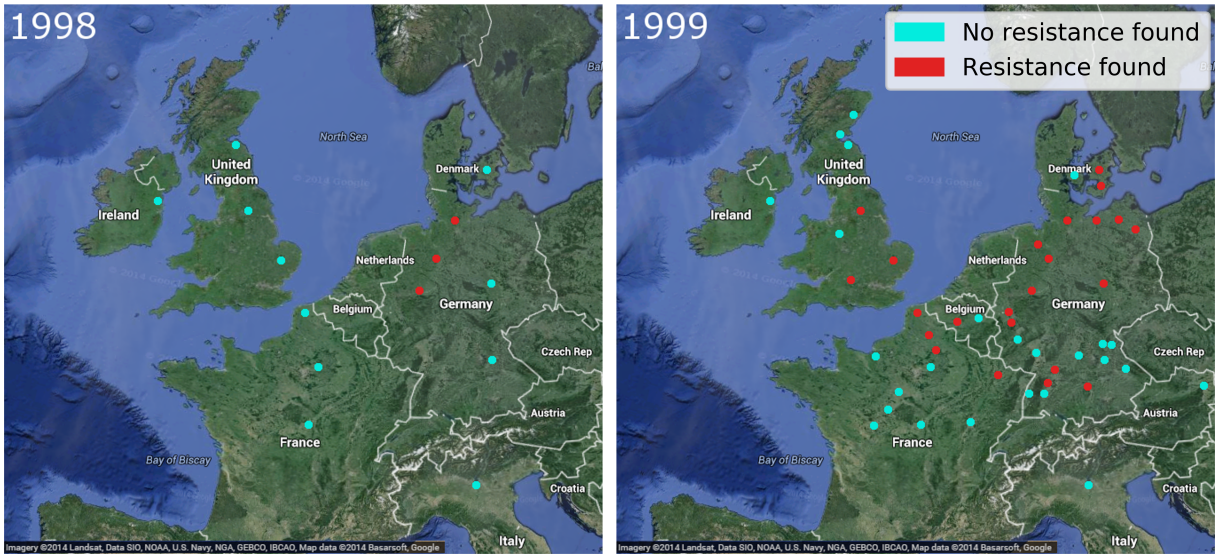

Figure 1.1: Spread of strobilurin resistance in *E. graminis* f. sp. *tritici* in 1998 and 1999. Red dots show survey sites where resistant isolates were found and red where none. Isolates were obtained by sampling airborne spores using a jet spore trap or collecting infected leaves in the field. Data from Chin *et al.* (2001), images from Google Earth.

1.2.4 Risk of resistance

Fortunately, there is variation in the extent to which resistance arises to different fungicides in the field. For example multi-site fungicides tend to provide more durable control as a greater degree of genetic change is required to overcome them. As an illustration of this, chlorothalonil has been heavily used since its introduction in 1964 with very little evidence of a decrease in effectiveness (Russell, 2005). A new range of SDHI fungicides released in 2003, however, already shows evidence of reduced effectiveness in multiple pathosystems and locations (Fungicide Resistance Management Committee, 2017). Although resistance risk is a spectrum, for our work we will broadly classify fungicides as low-risk and high-risk. A high-risk fungicide is one for which resistant strains already exist or are likely to emerge, whereas for low-risk the chance of significant resistance is negligible. Note that high-risk fungicides are often more specific in which organisms they target than low-risk. Increased specificity means higher doses can be used as the risk of detrimental off-target effects is reduced, and so high-risk fungicides are often more effective at controlling disease.

As well as the risk of resistance development varying between fungicides, resistance to different fungicides is not necessarily independent, which is termed cross-resistance (Brent & Hollomon, 2007b). Resistance to one fungicide leading to increased sensitivity to another is termed negative cross-resistance, and the opposite positive cross-resistance. Positive cross-resistance is considered to be more likely between fungicides that have similar chemical structures or target sites, and hence it is recommended to avoid spraying multiple fungicides within the same FRAC groups (Brent & Hollomon, 2007a). Cross-resistance depends on the exact mechanism of resistance, the SDHB H277Y mutation in *Alternaria*

alternata leads to negative cross-resistance between different SDHI fungicides whereas SDHC H134R leads to positive cross-resistance (Sierotzki & Scalliet, 2013).

1.2.5 Phases of resistance evolution

There are three distinct phases to fungicide resistance dynamics (van den Bosch & Gilligan, 2008): emergence, spread and persistence. Emergence refers to the situation where resistance is either non-existent in the population and must arise by *de novo* mutation or is very rare. During the emergence phase of resistance development, stochastic effects are important. Once a resistant strain has reached low to intermediate frequency it enters the spread phase. During this phase resistance spreads more or less exponentially and stochastic effects are of very little importance. Finally the resistant strain reaches the persistence phase where it reaches an equilibrium frequency, this may be fixation or coexistence with the sensitive strain depending on the exact system.

Before choosing which resistance management strategies to implement, it is important to know which phase the resistant strain is currently in as this will affect the relative performance of different options. The spread phase is by far the most well studied. Experimental studies on the emergence phase are very difficult due to the low frequencies of resistance that must be detected and the importance of rare events, but there are some modelling studies (Hobbelen *et al.*, 2014; Mikaberidze *et al.*, 2017). The persistence phase has relatively few studies (see Parnell *et al.* (2005, 2006); Shaw (2000)) as although it may be of academic interest, and has crossover with the ecological literature on species coexistence, the levels of resistance at which it occur are generally too high to be economically-sustainable.

1.3 Is resistance avoidable?

Fungicides apply a selection pressure to the fungal population which will lead to the propagation of resistance unless one of the following conditions is met.

1. There is an opposing selection pressure.
2. The effective population size for the fungus is small enough that genetic drift is more important than selection for the trait.
3. There is no generation of *de novo* resistance for selection to act on.

The first condition is usually described in terms of the “cost” of resistance. That is, the genetic changes that grant resistance also lead to or are genetically-linked to traits that reduce the fitness of the fungus. However these costs are not always present or obvious, with some studies showing significant costs and others finding no clear evidence (Milgroom, 2015). For example, some resistance-granting mutations of the succinate dehydrogenase

enzyme reduce its activity by over 90% but there is still not clear evidence that this reduces the fitness of resistant isolates (Sierotzki & Scalliet, 2013). In addition the costs of resistance can be reduced over evolutionary time. The MDR1 resistance phenotype in *B. cinerea* has been generated *de novo* convergently multiple times, but is now less common than the MDR2 phenotype which has evolved only a few times but carries significantly less cost to the organism (Walker *et al.*, 2017). Modelling work has suggested that when resistance is quantitative, a return to sensitivity can happen at most twice as slowly as the spread of resistance (Shaw, 1989a). On the other hand, Löcher *et al.* (1987) found significant returns toward fungicide sensitivity in a population of *Botrytis cinerea* between periods of dicarboximide application and major shifts toward resistance, suggesting heavy costs to resistance in this case.

To expand on the second point, an allele is effectively neutral when its selection coefficient is less than or equal to the inverse of the effective population size (Woolfit, 2009). However the effective population sizes of plant fungal pathogens are generally large (McDonald & Linde, 2002), and the effectiveness and widespread use of fungicides normally means that selection coefficients for fungicide resistance traits are large as well.

Thirdly, fungal pathogens typically produce large numbers of spores and have multiple asexual (and in some cases sexual) generations per growing season. The high rates of mutation and recombination this entails, and the relatively simple genetic changes needed to overcome high specificity fungicides means that the generation of novel resistance is unlikely to be a limiting factor in many cases. The exception to this are multi-site fungicides which may require multiple simultaneous genetic changes to be overcome.

It is very unlikely that fungicide resistance is avoidable in most cases (Russell, 2005). For the examples of fungicides that have provided durable control for a long time this is likely due to either the presence of costs or low rates of *de novo* resistance, or a combination of the two. Early attempts to manage fungicide resistance involved withdrawal of fungicides from specific use-cases in the hope that sensitivity would return but this was largely ineffective (Russell, 2005). There is some evidence that fungicide sensitivity will return after withdrawal of an active compound but this is not a general phenomenon and is expected to be slower than selection for resistance (Shaw, 1989a; Walker *et al.*, 2017).

1.4 Strategy and tactics

Throughout this thesis we will be using the terms strategy and tactic, and shall follow van den Bosch *et al.* (2015) in using these definitions with regard to fungicide resistance management,

Strategy What one is aiming to achieve.

Tactic How one implements the strategy.

Method Either a strategy or a tactic.

Some authors instead refer to individual methods of fungicide application as strategies or tactics depending on how likely they are to be effective, and although that definition is similar we shall avoid that usage (Shaw, 2006). Of course the exact definition of strategy and tactic is largely unimportant, as long as the definitions used are clear and consistent.

1.5 The fungicide resistance modelling literature

1.5.1 The early literature

Plant epidemiological modelling itself is a relatively young field, generally accepted to have been first formalised by van der Plank in the 1960s (van der Plank, 1963). Even with the availability of frameworks to model fungicide resistance spread, it was not originally considered to be a major problem by some with one pair of authors even stating (Georgopoulos & Zaracovitis, 1967),

> "The reported cases of tolerance to agricultural fungicides are very few and the knowledge accumulated hardly justifies a review."

They also stated,

> "That fungicide-tolerant strains of fungi are very rare is a good generalization. . . "

This is in stark contrast to the comments from a more recent paper on the closely-related problem of drug resistance in animal pathogens (Read *et al.*, 2011),

> "The eventual failure of drugs in the face of parasite evolution is now accepted as inevitable. . . "

1.5.2 Models without population dynamics

The first fungicide resistance models appeared a couple of decades later with the work of Kable & Jeffery (1980) and Delp (1980). This aligns with a generally increased estimation of the importance of fungicide resistance at that time, for example resistance to ethirimol in powdery mildew of barley and cucumber was known to be rapidly spreading (Russell, 2005). It is worth noting that there are a range of other study areas with findings applicable to fungicide resistance, such as ecological invasions and resistance to other pesticides, but we will focus on the fungicide resistance literature alone here.

These first fungicide resistance models in the 1980s were very simple, considering asexual pathogen strains that have identical population dynamics apart from their response to fungicide treatment. No elaboration is made on what form those population dynamics

take as it is unimportant to the model. In addition, very few assumptions are made about the effect of fungicides beyond that they kill a fraction of the fungal population, and that fraction is smaller for resistant (or "tolerant" in the language of Kable & Jeffery) strains. The practical nature of the work is emphasised by the fact that even this first paper considers the "escape fraction", the proportion of the fungal population that avoids fungicide due to incomplete spray coverage.

1.5.3 Models with exponential growth

Following on from Kable & Jeffery, further models of fungicide resistance dynamics were published by Skylakakis (1981), Levy *et al.* (1983), Josepovits & Dobrovolszky (1985), and Shaw (1989b). These models remained relatively simple but progressed from discrete-time models with unspecified population dynamics to explicitly consider continuous exponential growth of pathogen strains, with the effect of fungicide being to reduce the exponential growth rate rather than to outright kill a proportion of the population. These papers also put more detail into the model of the fungicides themselves, considering more realistic dynamics of fungicide concentration and focussing relatively heavily on assumptions about the way in which different fungicides might interact.

1.5.4 Models including additional epidemiology

Alongside the relatively simple and often analytically-tractable models of fungicide resistance, more complex simulation models started to appear as well (Josepovits, 1989; Levy & Levy, 1986; Milgroom & Fry, 1988). These focussed more on practical application rather than theoretical understanding and brought in features such as weather, host tissue availability limiting infection rates and decay of applied chemicals. As such they were often parameterised to match particular pathosystems.

Following more general trends in plant disease epidemiology (Cunniffe *et al.*, 2015b; Gilligan & van den Bosch, 2008; Madden, 2006), compartmental models of fungicide resistance also began to be developed. Initial work was relatively simple, focussing largely on the implications of a cost of resistance on the long-term persistence of fungicide-resistant and -sensitive strains (Gubbins & Gilligan, 1999). Further work showed how the additional complexities of spray heterogeneity (Parnell *et al.*, 2005, 2006; Shaw, 2000) and realistic responses to the fungicide dose (Hall *et al.*, 2004, 2007) could be treated in a compartmental model. These initial compartmental models were generic, focussing on broad principles applicable to all systems.

The modern fungicide resistance modelling literature is characterised by the use of complex pathosystem-specific simulation models of resistance evolution (Hobbelen *et al.*, 2011a,b, 2013, 2014; Kitchen *et al.*, 2016; van den Berg *et al.*, 2013, 2016). These models are carefully fitted to field data and should give reasonable predictions of epidemic dynamics

under different fungicide application regimes. However their complexity means that elucidating the mechanisms driving the results is more difficult, and so models with a greater focus on understanding rather than biological accuracy are still important (Mikaberidze *et al.*, 2014, 2017).

1.6 Selection and fitness

There are a number of varying definitions of fitness in the literature but all describe the ability of individuals or groups to survive and reproduce in their environment (Orr, 2010a). Tied closely to the concept of fitness is the selection coefficient, s, which is used as a measure of differences in fitness and thus strength of selection for a given genotype or allele. The selection coefficient too can be defined in different ways, depending on the evolutionary model used and the aim of the analysis. The aim of of this section is explain the form of the selection coefficient used in later reasoning. For brevity, in the following discussion we shall use genotype to refer both to the genotype itself and the sub-population carrying that genotype. In addition we will be assuming haploid genetics and clonal reproduction but the core analysis can be readily extended to include more complicated systems.

Fitness can be described in terms of absolute fitness, W, or relative fitness, w. Absolute fitness describes the fitness of a particular genotype in of itself, *e.g.* its expected probability of survival or expected number of offspring. Relative fitness normalises this absolute fitness in some way to the rest of the population. There is no standard measure of fitness for filamentous fungal because the measurement of their survival and reproduction can be obscured by difficulties in defining what an individual is, and also because their life cycles and genetics seem complicated in comparison to animals and plants (Pringle & Taylor, 2002). However the use of Malthusian fitness, or intrinsic rate of increase, is popular in the fungicide modelling literature (van den Bosch & Gilligan, 2008) despite the fact that it is rarely recorded experimentally (Pringle & Taylor, 2002). The way in which absolute fitness is defined can have important effects on the behaviour of a model (Wu *et al.*, 2013), although it will be guided by the exact biology being modelled. For example Kable & Jeffery (1980) defines fitness in terms of survivorship; how likely an individual of a given genotype is to survive and contribute to the next generation. Skylakakis (1981), on the other hand, defines fitness in terms of the per capita growth rate of the genotype. This leads to conflicting predictions, Kable & Jeffery (1980) predicts that decreasing the fitness of all genotypes by the same factor will have no effect on selection whereas Skylakakis (1981) predicts that this will slow selection. These differences in definition then lead directly on to differing conclusions between the two papers when comparing different disease management tactics. This highlights the importance of thinking carefully about definitions of fitness and selection when constructing a model.

We favour a definition of the selection coefficient that leads to $s > 0$ meaning positive

Box 1.1: Deriving the selection coefficient

For a population with n asexually-reproducing strains and overlapping generations we define the absolute fitness of the i^{th} strain as the Malthusian fitness (intrinsic rate of increase). We do not assume exponential growth and hence this is not necessarily constant.

$$W_i(t) = \frac{\mathrm{d}N_i(t)}{\mathrm{d}t} N_i(t)^{-1}\,, \tag{1.1.1}$$

where $N_i(t)$ is the number of individuals of the i^{th} genotype at time t. The change in the frequency of the i^{th} strain $p_i(t)$ is then given (Crow & Kimura, 1970) by

$$\frac{\mathrm{d}p_i(t)}{\mathrm{d}t} = (W_i(t) - \bar{W}(t))p_i(t) \tag{1.1.2}$$

$$\bar{W}(t) = \sum_{i=0}^{n} p_i(t)W_i(t) \tag{1.1.3}$$

$$p_i(t) = \frac{N_i(t)}{\sum_{j=0}^{n} N_j(t)}\,. \tag{1.1.4}$$

If there are only two strains (denoted R and S) then this simplifies to,

$$\frac{\mathrm{d}p_R}{\mathrm{d}t} = s_R(t)p_R(t)(1 - p_R(t)) \tag{1.1.5}$$

$$\begin{aligned} s_R(t) &= \frac{\mathrm{d}}{\mathrm{d}t}\log\left(\frac{N_R(t)}{N_S(t)}\right) \\ &= \frac{\mathrm{d}N_R(t)}{\mathrm{d}t} N_R(t)^{-1} - \frac{\mathrm{d}N_S(t)}{\mathrm{d}t} N_S(t)^{-1}\,. \end{aligned} \tag{1.1.6}$$

Under the assumption of exponential growth this gives the selection coefficient as the difference in the intrinsic growth rates of the strains, which is the definition used in the fungicide resistance literature (Leonard & Fry, 1989; van den Bosch *et al.*, 2014a),

$$s = r_R - r_S\,, \tag{1.1.7}$$

where r_R and r_S are the intrinsic growth rates of the fungicide-resistant and sensitive strains respectively. This definition of the selection coefficient is convenient as it is independent of the state of the system and can be calculated from typical epidemiological parameters. For cases where the selection coefficient is not constant in time then the appropriate metric is the mean selection coefficient over the time period T_1 to T_2 (Crow & Kimura, 1970; Leonard & Fry, 1989),

$$\bar{s} = \frac{1}{T_2 - T_1}\int_{T_1}^{T_2} s(t)\mathrm{d}t \tag{1.1.8}$$

$$\int_{T_1}^{T_2} s(t)\mathrm{d}t = \log\left(\frac{N_R(T_2)}{N_S(T_2)}\right) - \log\left(\frac{N_R(T_1)}{N_S(T_1)}\right)\,. \tag{1.1.9}$$

selection, $s < 0$ negative and $s = 0$ referring to no expected change in allele frequencies (see box 1.1). Definitions of the selection coefficient need not lead to this relationship, but this definition is not unused in the literature (Crow & Kimura, 1970; Fraïsse *et al.*, 2014; Orr, 2010b).

1.7 The governing principles of resistance evolution

Recently van den Bosch *et al.* reviewed and evaluated the effectiveness of some simple governing principles for predicting the impact of fungicide applications on resistance development during the spread phase (van den Bosch *et al.*, 2014a). These principles were first introduced in the 1980s in Staub & Sozzi (1983) and Milgroom & Fry (1988), and has proved a good rule of thumb for estimating the qualitative impact of a fungicide spray program on resistance development (van den Bosch *et al.*, 2014a). These governing principles area based on multiplying the selection coefficient for resistance (s) by the exposure time to fungicide (T). Decreasing this quantity leads to less rapid resistance spread. This implicitly assumes that the selection coefficient is constant over the time of selection, and so we generalise these principles to consider a quantity we term the cumulative selection coefficient,

$$\sigma = \int_0^T s(t)\mathrm{d}t\,. \tag{1.1}$$

Note that it is clearly equivalent to take the integral of the mean selection coefficient or the selection coefficient as a function of time. Clearly one can slow the spread of fungicide resistance by reducing one or both of T and $s(t)$, which is the way in which most typically suggested anti-resistance tactics function. Given a definition of the selection coefficient we can make more specific predictions, for example using equation 1.1.7 we can see that there are three main strategies to slow the spread of resistance (van den Bosch *et al.*, 2015),

Strategy 1 Decrease the growth of both sensitive and resistant strains

Strategy 2 Decrease the growth of the resistant strain relative to the sensitive strain

Strategy 3 Reduce the exposure time to fungicide

These strategies will be referred to by number in the next section.

1.8 Methods for managing fungicide resistance

One of the simplest and yet most controversial tactics for reducing the rate of fungicide resistance development is a reduction in dose (van den Bosch *et al.*, 2011). This functions by reducing the difference in fitness between resistant and sensitive strains and thus the selection coefficient (Strategy 2). One of the reasons that this tactic has proved controversial

is that it is contrary to commonly-held wisdom in the closely-related fields of pesticide resistance in herbicides, insecticides and rodenticides which generally advise the use of high doses combined with refugia where no pesticide is used. The high dose and refugia tactic focusses on reducing the survival of partially resistant heterozygotes, and maintaining a breeding population of sensitive homozygotes. However unlike weeds, insects or rodents; fungal or oomycete pathogens are generally haploid, clonal dikaryons or clonal diploids and so this mechanism is irrelevant (van den Bosch *et al.*, 2011). High doses are also favoured in managing antibiotic resistance, with a focus on killing of partially-resistant strains and reducing the population mutation rate through population suppression. It is possible that higher doses may reduce the emergence of fungicide resistance in the same way; however the evidence for the effectiveness of this strategy even in antibiotic resistance management is somewhat lacking (Day & Read, 2016; Read *et al.*, 2011). One study suggests that fungicide applications may reduce the effective population size of *B. cinerea* by an order of magnitude (Walker *et al.*, 2017), but it is not clear that this is sufficient to offset the increased selection pressure. In addition, suppressing the pathogen population size may allow stochastic local extinction, although this seems unlikely given the difficulties of obtaining full spray coverage and the long-distance dispersal of many foliar fungal pathogens. On the other hand, there is evidence in bacteria that mutation rates are increased under chemical stress (van den Bosch *et al.*, 2011) and emerging evidence that this may also be the case in some fungal plant pathogens (Amaradasa & Everhart, 2016), which would favour lower doses for suppression of resistance emergence. Finally, spread of resistance may be slowed under a high dose strategy if resistance to the fungicide is partial and the dose-response curves for the resistant and sensitive strains converge at higher doses (the reverse of Strategy 2). This may be especially important if partially resistant strains can act as evolutionary “stepping stones” to full resistance. However overall, the majority of the experimental and modelling literature conclude that decreasing the dose of the fungicide applied will decrease the spread of fungicide resistance (van den Bosch *et al.*, 2011).

To offset the loss in disease control associated with reducing dose, multiple fungicides can be applied simultaneously. Mixture can provide an additional benefit on top of the effect of a reduction in dose. Assuming there is no cross-resistance between the mixed fungicides, then mixture will suppress the growth of both the resistant and sensitive strains and reduce resistance spread even further (Strategy 1).

In the same way as mixture compensating for a reduction in dose, alternation can compensate for a reduction in the number of sprays. Alternation refers to the situation where sprays of different fungicides are alternated over the course of a spray program. If there is no cross-resistance between the alternated fungicides, and they decay such that significant concentrations of each chemical do not overlap, then the only benefit for resistance management that alternation supplies is due to a reduction in the number of sprays of each fungicide (Strategy 3).

Reducing the number of sprays of a fungicide will reduce the time of exposure to selection (Strategy 3). There are few studies on this uncontroversial idea, but all those that do exist agree that increasing the number of sprays increases the selection for resistance (van den Bosch *et al.*, 2014a). There is a further effect of increasing the number of sprays beyond exposure time due the effect of dose-splitting. Fungicide dose-response curves are often concave; they experience diminishing returns with increased dose. This means that reducing the dose by a factor will reduce the effect on the pathogen by a smaller factor. Thus splitting the same total dose of fungicide over a greater number of sprays actually increases selection for resistance, as the difference in growth rates between the resistant and sensitive strains is decreased by a smaller factor than the time of exposure to fungicide (Strategy 3 against the reverse of Strategy 1).

The above tactics are simple and easy to reason about with the governing principles. There are however a number of more complicated tactics beyond the scope of the simple model of resistance development implicit within the governing principles. If resistance development to multiple fungicides is not independent due to cross-resistance, then it may be possible to apply combinations of fungicides in alternation or mixture that provide greater benefit than the governing principles alone would suggest. Other more complex tactics rely on spatial heterogeneity of fungicide application; for example out of phase rotations in adjacent fields. There are a large number of potential tactics utilising spatial heterogeneity which can generally only be explored with modelling studies (Hobbelen *et al.*, 2013; Parnell *et al.*, 2005, 2006).

1.9 The work in this thesis

In this thesis we will evaluate the performance of a range of fungicide application tactics through mathematical modelling. We first start by introducing the models we will be using, and explaining some of the reasoning behind their particular construction. We then investigate the relative performance of mixture and alternation, taking into account both the effect on resistance evolution and crop yield. Having generated some predictions about the performance of mixture and alternation, we then investigate the robustness of those predictions to a number of features of the models used. Having used the governing principles throughout to explain the performance of the tactics, we then continue on to test the predictive power of the principles more rigourously. Finally we will investigate the effect of changing the timing of fungicide sprays, both on the impact of that single spray and as part of larger application regimes.

Chapter 1 Summary

- Fungicides are pesticides used to control fungal disease of crops.
- Use of fungicides encourages the evolution of fungicide resistance and a loss in the effectiveness of disease control and yield.
- There is variation in the risk of resistance evolution to different fungicides.
- We consider fungicides to either be low-risk or high-risk for the purposes of this thesis.
- There are a simple set of governing principles for predicting the impact of particular fungicide application tactics on resistance evolution.

2

Model selection and development

> *Chemical industry and plant breeders have forged fine tactical weapons, but only epidemiology sets the strategy.*
>
> J. E. van der Plank, *Plant Diseases: Epidemics and Control*, 1963

2.1 Introduction

A number of mathematical models are used throughout the course of this work; we introduce and summarise them in this chapter. All models used are in the form of systems of ordinary differential equations (ODEs) which describe a compartmental epidemiological model, a modelling framework originally introduced by Kermack & McKendrick (1927). All models used are continuous in both time, and compartment size. The models vary in complexity and the exact biology that they attempt to capture. Note that the notation used here does not necessarily match that used in the original papers the models were sourced from. This aids in identifying the common features between models and avoids reusing some symbols representing key concepts in other parts of the thesis. The default parameter values for each model can be found in Appendix A.

2.2 Model for the effect of fungicides

2.2.1 Protectant and eradicant effects

We model fungicides as affecting the rates of particular life-cycle processes of the pathogen and following the recent literature (*e.g.* see Hobbelen *et al.* (2011b); van den Berg *et al.* (2016)) we model protectant activity as reducing the infection rate and eradicant as reducing the rate at which tissue becomes infectious after infection. In the framework of a classic SEIR (Susceptible, Exposed, Infectious, Removed) model protectant activity reduces the rate of the S to E transition and eradicant of the E to I.

2.2.2 Dose-response curves

To determine the degree to which a fungicide slows life-cycle processes we use the concept of a dose-response curve. Dose-response curves are used throughout the fungicide literature, both experimental and theoretical. They relate how some effect of the fungicide is related to some measure of its dose. There is variation in the exact definition used, for example a grower may use it to describe the relationship between sprayed dose and reduction in visible disease in the crop at harvest, whilst a laboratory scientist may relate the concentration of fungicide in a growth medium to the rate of mycelial growth. For our purposes, a dose-response curve is the relationship between the concentration of fungicide on the leaf and the degree to which a particular life-cycle process is slowed. We follow the theoretical literature in assuming that the dose-response curve for the protectant and the eradicant activity of a fungicide are the same.

There are two commonly-used simple functional forms used for determining the effect of a fungicide at a particular dose (C), exponential and Michaelis-Menten (figure 2.1). The former has the form,

$$\epsilon(C) = \omega(1 - \mathrm{e}^{-\theta C}), \tag{2.1}$$

and the latter

$$\epsilon(C) = \frac{V_{max} C}{k_m + C}. \tag{2.2}$$

The Michaelis-Menten form is more firmly grounded in reality with both parameters having biological interpretation and is derived from molecular kinetics. The parameter V_{max} is the asymptotic maximum possible effect and k_m is the dose at which half the maximum effect is achieved. The parameter ω has the same interpretation as V_{max} but θ is harder to assign biological meaning, despite having a similar effect to k_m.

Both functional forms can be fit to field data with similar levels of confidence, and so which is used is largely down to which features of the dose-response curve are required. For example, it can sometimes be convenient to be able to integrate the dose-response functions with respect to time as this gives a measure of the total effect imposed on the pathogen over a time period. Depending on the time-profile of the fungicide dose this may or may not analytically soluble, but for the typical case of exponential decay the Michaelis-Menten form is soluble whilst the exponential form requires numerical approximation. This integral under the Michaelis-Menten model (equation 2.2) has the form

$$\int \frac{V_{max} C \mathrm{e}^{-\delta t}}{k_m + C \mathrm{e}^{-\delta t}}, \mathrm{d}t \tag{2.3}$$

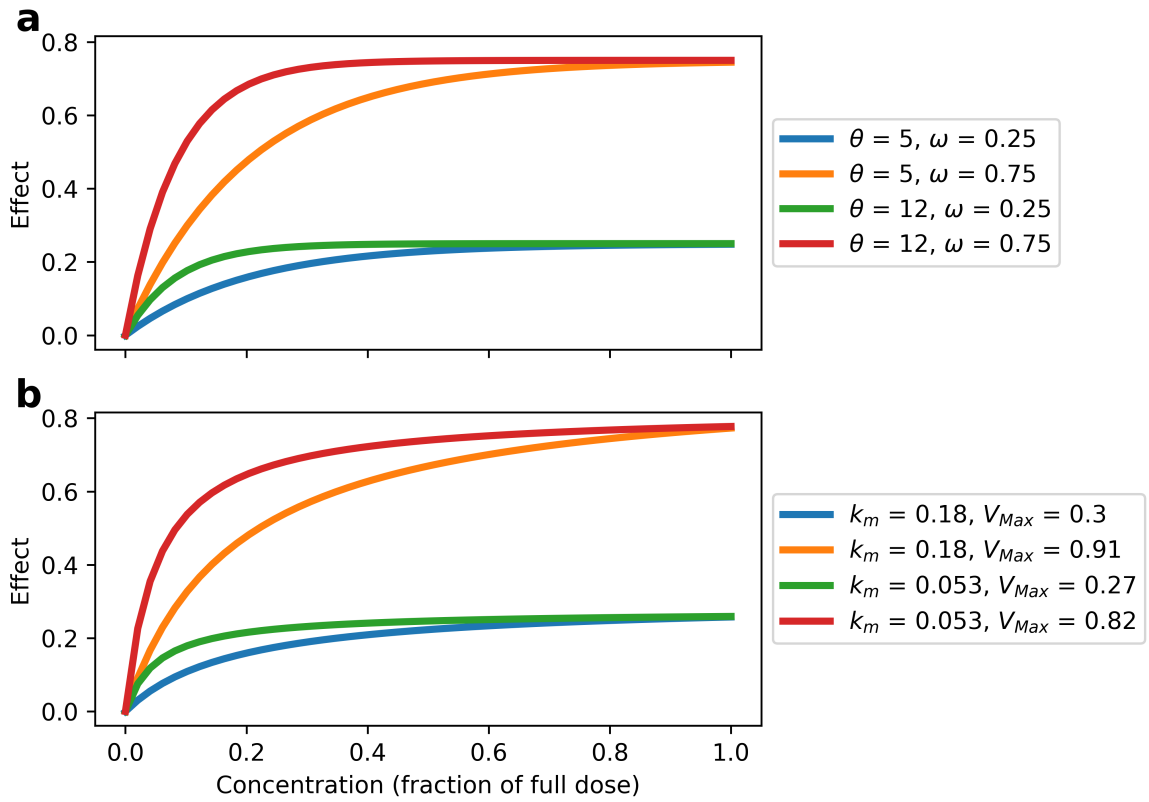

Figure 2.1: Dose-response curves under the **a)** exponential and **b)** Michaelis-Menten models for a range of parameter values. The Michaelis-Menten parameters are chosen to produce curves similar to those of the exponential model. The effect is the complement of the multiplier on the affected life cycle parameter and the concentration is given as a fraction of the full label dose. The parameters ω and V_{Max} control the height of the horizontal asymptote and θ and k_m how quickly the asymptote is approached.

and under the exponential model (equation 2.1)

$$\int \omega(1 - e^{-\theta C e^{-\delta t}})\, dt\,, \tag{2.4}$$

where δ is the exponential decay rate.

2.2.3 Synergy

When considering mixture of chemicals it is important to consider synergy between the constituent parts. Synergy refers to the interaction between effects of different chemicals, such that the effects of the chemicals together is greater than expected by each chemical working independently (Berenbaum, 1989). Synergy is generally defined by comparing the effect of the mixture to a null case of independent action; the difficulty of defining this null case and different models of synergy is covered in detail in Shaw (1989b) and Geary (2013). For our work we focus on the case of independence of effect, as although there are a number of examples of synergy of fungicide effects (Cohen & Levy, 1990; Shaw, 1989b)

choosing a model for synergy is non-trivial (Geary, 2013). Assuming independence allows us to sidestep a number of issues relating to defining synergy, and instead need only choose between the two main models for describing lack of synergy; Loewe Additivity and Bliss Independence (Geary, 2013). It is also typical in the modern fungicide resistance modelling literature to assume independence of effect (Hobbelen *et al.*, 2011b, 2013; Mikaberidze *et al.*, 2014; van den Berg *et al.*, 2016; van den Bosch *et al.*, 2014b).

The exact mathematical form these models take depends on the dose-response curve being used. Loewe Additivity is based on the concepts of sham mixture and drug dose equivalence. Firstly a drug should not synergise with itself; mixing two half-doses of the same drug should have exactly the same effect as a full dose. Secondly, we can treat one drug as acting as a dilution of another, though the relationship between the dose of one drug to another may not be constant with dose. Bliss Independence is instead based on an argument stating that drug effects are due to probabilistic processes and so zero interaction can be modelled as independence of these processes. There are arguments for both of these models (see Geary (2013) for review) but using exponential dose-response curves avoids much of this as both of the above synergy models lead to the same form for calculating the effect of mixture. The exponential model of independence assumes that the complement of the effect of a mixture is the product of the complements of the components,

$$(1 - \epsilon(C_1, C_2)) = (1 - \epsilon_1(C_1))(1 - \epsilon_2(C_2))\,. \tag{2.5}$$

The Michaelis-Menten form however requires an explicit choice of synergy model as the different choices lead to different forms for the effect of mixture. Ultimately we choose to use the exponential form because although it decreases analytical tractability it reduces the number of assumptions we must make about the effects of chemicals in mixture, which is not the main focus of our work.

2.2.4 More complex dose-response curves

Although the exponential form is capable of representing a simple dose-response curve with diminishing returns, it cannot recreate the shape of more complicated dose-response curves which have occasionally been found experimentally; for example the multiphasic dose-response of *Ustilago avenae* to mycobutanil seen in Koller & Wubben (1988). It would be simple however to use a different dose-response in our models to check the robustness of our results, for example a multiphasic Hill model could be used to cover a range of possible response forms (Di Veroli *et al.*, 2015). However such complex dose-response curves are not typical in the theoretical fungicide resistance literature and would require more data to fit.

2.2.5 Fungicide concentration

As well as relating the concentration of a fungicide to its effect we must also model the dynamics of the fungicide concentration over time. This varies between models and thus is described with each specific model, but in in general we assume that fungicide sprays lead to an instantaneous increase in concentration followed by exponential decay of the chemical.

2.3 Multiple pathogen strains

For each model we follow epidemics caused by two pathogen strains, one resistant to fungicide (denoted R) and one sensitive (S). We almost always consider the use of only two fungicides, one high-risk and one low-risk, the concentrations of which are denoted C_H and C_L respectively. The effects of the fungicides at a particular dose are then denoted $\epsilon_H(C_H)$ and $\epsilon_L(C_L)$, indicating that in general the dose-response parameters vary between different fungicides. In chapter 4 we briefly consider the case of using two high-risk fungicides. In all cases the two strains act identically apart from the the high-risk fungicide having no effect on the resistant strain, and the resistant strain starting at much lower frequency than the sensitive. Any compartment which has both fungicide-resistant and -sensitive strains will be differentiated by subscripts with the appropriate letter.

2.3.1 Partial resistance

The models are readily generalisable to n strains, and the same generalisation opens up the models to consider partial resistance. Each of the n strains is assigned a characteristic value for a resistance parameter r for each fungicide modelled. The value of r for a given strain and fungicide directly modulates the dose-response curve. For comparison with the full resistance case, r is limited to the range [0, 1] where 0 represents full susceptibility and 1 full resistance.

The effect of r might be applied to the maximum effect or curvature of two-parameter dose-response curves, Type 1 and Type 2 resistance respectively in the terms of Mikaberidze *et al.* (2017). For full resistance Type 1 and Type 2 resistance are equivalent, but differ in their effects on resistance development when partial resistance is considered (Mikaberidze *et al.*, 2017). There are many functional forms one might choose for the effect of r on the dose-response curve parameters but, since the modeller is free to choose the number of strains, their r values and their initial frequencies, the choice is unimportant and so we opt for the simplest choice taking,

$$\rho = 1 - r\,, \tag{2.6}$$

where ρ is a factor applied to the relevant parameter of the dose-response curve.

2.4 Septoria leaf blotch of UK winter wheat

2.4.1 The pathosystem

Wheat is the most widely-grown crop in the world, and is the second most important global food crop behind rice (Fones & Gurr, 2015). The main disease of wheat in Europe is septoria leaf blotch, caused by *Zymoseptoria tritici* (synonyms: *Mycosphaerella graminicola*, *Septoria tritici*). Severe epidemics can lead to yield losses of up to 50% (Fones & Gurr, 2015), as well as affecting grain quality (McKendry *et al.*, 1995). Septoria alone accounts for around 70% of annual EU fungicide use (Fones & Gurr, 2015). In spring 2012 almost 60% of United Kingdom cereal farmers indicated use of at least one SDHI-containing product per season, only 2 seasons after the first introduction of these compounds (Sierotzki & Scalliet, 2013). With such heavy use of fungicide, it is unsurprising that many key fungicides effective against septoria show widespread resistance.

As well as being economically important, septoria of UK winter wheat is also well studied and so provides a good case study for our work. We use two main models to investigate this pathosystem. These two models in particular are used as they arguably represent the most advanced models available in the fungicide resistance modelling literature and have been parameterised to field data. The first model is somewhat less complex than the second, which eases understanding and allows closer comparison to analytic work.

2.4.2 FiveLeaf model

Model description

The first model we use is based on a series of recent papers (Hobbelen *et al.*, 2011a, 2013, 2014). It was first introduced as a model of powdery mildew (*Blumeria graminis* f. sp. *hordei*) on spring barley (*Hordeum vulgare*) (Hobbelen *et al.*, 2011b) but was later reparameterised to represent septoria on wheat (Hobbelen *et al.*, 2011a). The model explicitly tracks the infection status of leaves 1 - 3 of the plant, counting downward from the flag leaf. Lower leaves are represented as the source of primary inoculum for epidemics on the upper leaves, but are otherwise not considered by the model (figure 2.2).

The measure of time in the model is degree-days accumulated above 0 °C. The average daily temperature is assumed to be 15.2 °C, matching that of the average growing season temperature in Cambridgeshire from 1984 to 2003 (Hobbelen *et al.*, 2011a). This measure of time is used to attempt to include the effect of weather conditions on crop physiology and disease progression.

The unit of measurement for leaf tissue in the model is the leaf area index (LAI). LAI is a standard physiological unit and is the ratio of leaf area to area of ground covered. The total

LAI across leaves 1 - 5 (A) grows initially monomolecularly,

$$\frac{\mathrm{d}A}{\mathrm{d}t} = r(k - A)\,, \tag{2.7}$$

and is very close to its maximum value (k) at GS39 on the Zadoks scale. This total area is split into susceptible (S), latently-infected (E), infectious (I) and dead (R) tissue. Septoria is generally classified as a hemibiotroph, although this is contested (Sanchez-Vallet *et al.*, 2015), in which the pathogen first feeds on living host tissues before switching to a necrotrophic mode where it kills and feeds off the remains of host tissue. To model this, on infection, first tissue enters a latent compartment which is not infectious and is still alive before passing into the infectious and dead compartment. Infection can be caused by either primary inoculum on the lower leaves (P) or secondary on the upper leaves (I), and is assumed to follow frequency-dependent transmission (sometimes called true mass action). Frequency-dependent rather than density-dependent transmission is a better fit for this system for as the total leaf area index increases, the plant is growing larger and tissues do not necessarily get any closer together (McCallum *et al.*, 2001).

At GS61 the living tissues (S and E) begin to senesce with rate

$$\Gamma(t) = \begin{cases} 0.005\left(\dfrac{t - T_{GS61}}{T_{GS87} - T_{GS61}}\right) + 0.1\mathrm{e}^{-0.02(T_{GS87}-t)}, & \text{for } t >= T_{GS61} \\ 0, & \text{otherwise} \end{cases}\,, \tag{2.8}$$

where $T_\bullet$ represents the time of particular Zadoks growth stages. It is assumed that the presence of disease has no effect on growth and senescence of still-living (S and E) tissues.

Fungicide applications are modelled as discrete changes in the state variables representing fungicide concentration. The key fungicide spray timings for septoria control in the UK are the T1 (at around GS32) and the T2 (at around GS39), with T0 and T3 used less commonly (Paveley *et al.*, 2014). Therefore we focus on applications just at the two main timings.

The total leaf area index is defined as,

$$A = S + E_R + E_S + I_R + I_S + R\,, \tag{2.9}$$

and the full set of equations for this model are

$$\frac{\mathrm{d}S}{\mathrm{d}t} = r\,(k - A) - \Gamma S_i\,(t) \tag{2.10}$$

$$- \beta\frac{S}{A}\Big(\Big(1 - \epsilon_L\,(C_L)\Big)\,(I_R + P_R) + \Big(1 - \epsilon_L\,(C_L)\Big)\Big(1 - \epsilon_H\,(C_H)\Big)\,(I_S + P_S)\Big) \tag{2.11}$$

$$\frac{\mathrm{d}E_R}{\mathrm{d}t} = \beta\frac{S}{A}\Big(1 - \epsilon_L\,(C_L)\Big)\,(I_R + P_R) - \Gamma\,(t)\,E_R - \gamma E_R \tag{2.12}$$

$$\frac{\mathrm{d}E_S}{\mathrm{d}t} = \beta\frac{S}{A}\Big(1 - \epsilon_L\,(C_L)\Big)\Big(1 - \epsilon_H\,(C_H)\Big)\,(I_S + P_S) - \Gamma\,(t)\,E_S - \gamma E_S \tag{2.13}$$

$$\frac{\mathrm{d}I_R}{\mathrm{d}t} = \gamma E_R - \mu I_R \tag{2.14}$$
$$\frac{\mathrm{d}I_S}{\mathrm{d}t} = \gamma E_S - \mu I_S \tag{2.15}$$
$$\frac{\mathrm{d}R}{\mathrm{d}t} = \mu\,(I_R + I_S) + \Gamma(S + E_R + E_S) \tag{2.16}$$
$$\frac{\mathrm{d}P_R}{\mathrm{d}t} = -\nu P_R \tag{2.17}$$
$$\frac{\mathrm{d}P_S}{\mathrm{d}t} = -\nu P_S \tag{2.18}$$
$$\frac{\mathrm{d}C_H}{\mathrm{d}t} = -\delta_H C_H \tag{2.19}$$
$$\frac{\mathrm{d}C_L}{\mathrm{d}t} = -\delta_L C_L\,. \tag{2.20}$$

The model is run over multiple seasons by resetting all state variables back to their initial conditions. The only change between seasons is the frequency of the resistant strain in the primary inoculum. This is set to be the same as the frequency of resistance in the infectious tissue at the end of the previous season. For the *n*th season,

$$\phi = \frac{I_R^{n-1}(T_{GS87})}{I_R^{n-1}(T_{GS87}) + I_S^{n-1}(T_{GS87})} \tag{2.21}$$
$$I_R^n(T_{GS32}) = \phi\psi \tag{2.22}$$
$$I_S^n(T_{GS32}) = (1-\phi)\psi\,, \tag{2.23}$$

where the *n* superscripts represent the value of the state variable in a given season and ψ is the total amount of primary inoculum when the simulation starts.

The parameters of this model to a large extent already have established values in the literature, and so where possible we used those values directly (appendix A.1.1).

In order to estimate the yield produced by the wheat crop at a given level of disease we follow Hobbelen *et al.* (2011b) in taking the integral of photosynthetically-active leaf area index between GS61 and GS87,

$$\text{Yield} = \int_{T_{GS61}}^{T_{GS87}} (S + E_S + E_R)\,\mathrm{d}t\,. \tag{2.24}$$

This has been shown to be both correlated and mechanistically linked with yield (Gooding & Dimmock, 2000; Waggoner & Berger, 1987).

Increasing the number of leaves

The model as described here starts simulation earlier (244 degree-days) and tracks two further leaves compared to the model we have adapted it from (Hobbelen *et al.*, 2011b). We made this change in order to avoid an edge effect that we identified in the original model.

In the model primary and secondary infection are modelled separately, and the amount

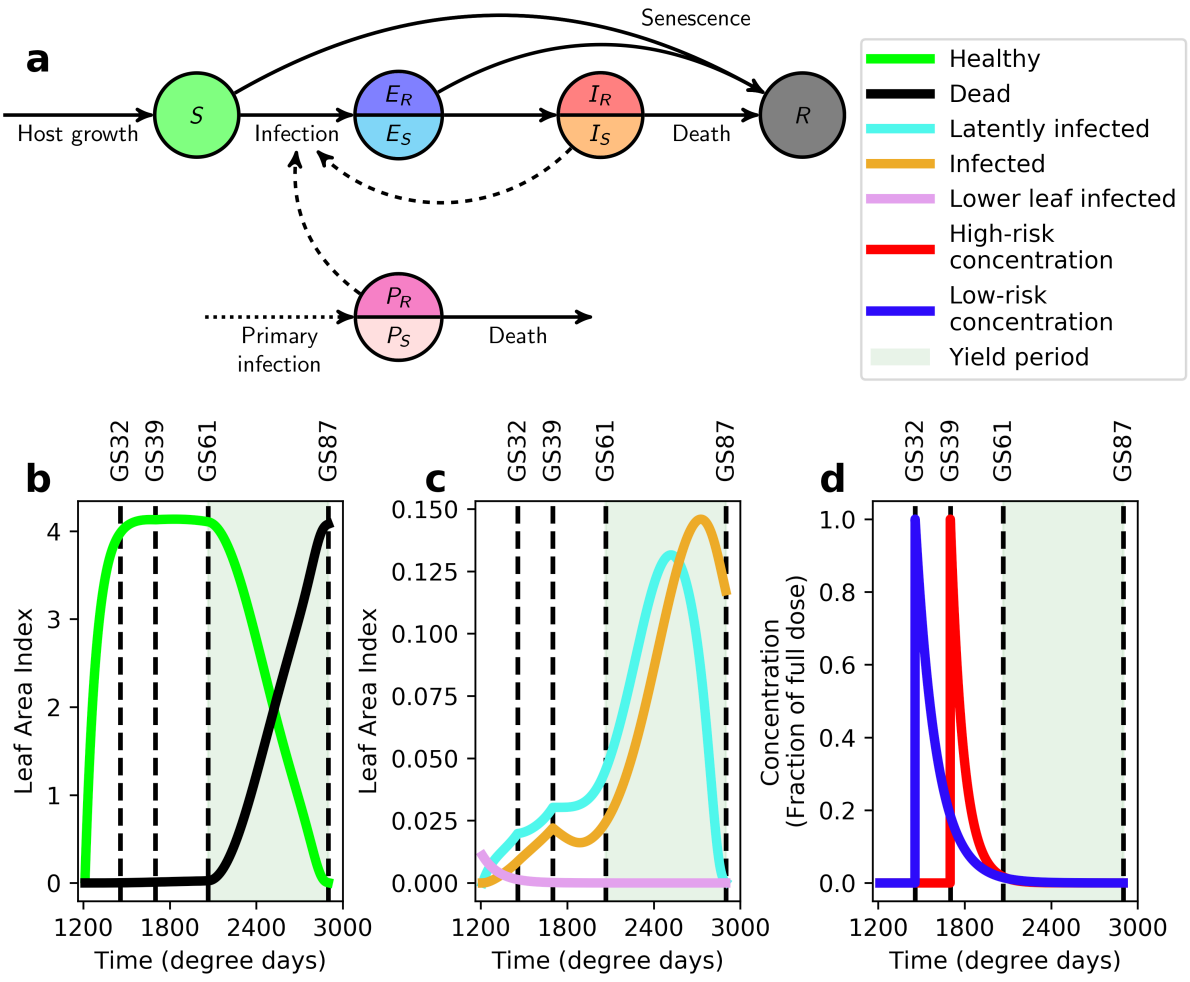

Figure 2.2: The structure and dynamics of the FiveLeaf septoria model. **a)** A schematic of the model structure. Circles are epidemiological compartments (split into two where necessary for each pathogen strain), solid lines are transitions, dashed lines represent effects on the rates of transitions and the dotted line shows the point of initial infection. **b - d)** The dynamics of the model over a single season when a full dose of the low-risk is applied at GS32 and of the high-risk at GS39. **b)** The amount of healthy and dead tissue over time. **c)** The amount of primary inoculum and infected tissue over time. **d)** The amount of fungicide over time. The default parameter values for this modelled are given in appendix A.1.1.

of primary infection decreases over the season. This means that the relative rates of primary and secondary infection shift over time from primary infection being dominant at the beginning of the season to secondary dominating toward the end.

The first fungicide application (T1) in the model occurs at GS32, the same time as the emergence of leaf 3. In the original formulation of the model (Hobbelen *et al.*, 2011a) this means the first spray occurs when all infection is due to primary inoculum, and the second at GS39 when secondary infection is much more important. The per capita pathogen growth rate has a large impact on the selective pressure imposed by a fungicide spray (according to the governing principles, see later chapters), and this rate approaches infinity as $t \to T_{GS32}^{+}$ in the model as originally posed with three leaf layers. The earlier spray therefore had a

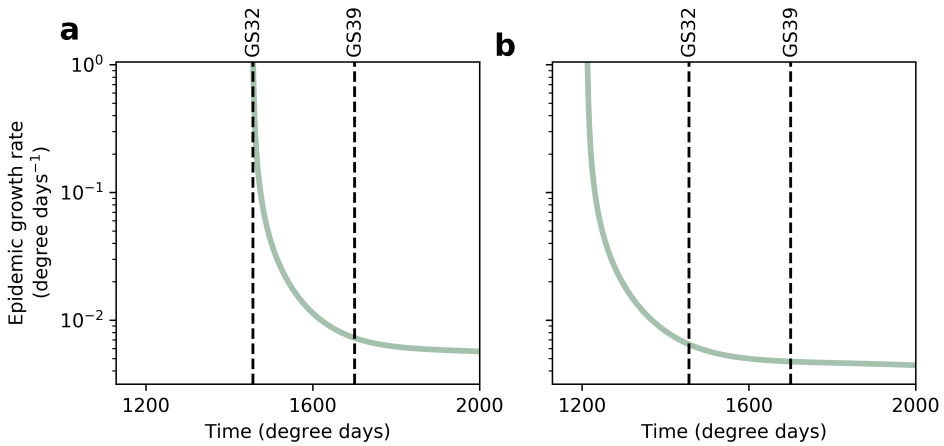

Figure 2.3: The epidemic growth rate over time when no fungicide is applied, in the model **a)** when only leaves 1-3 are modelled explicitly and **b)** when leaves 1-5 are modelled. The epidemic growth rate is defined as $\frac{\mathrm{d}I(t)}{\mathrm{d}t}I(t)^{-1}$.

much larger effect on the development of resistance than the later. This was decided to be an artefact of the model rather than an accurate recreation of reality, and so the model start time was moved two phyllochrons earlier to avoid the edge effect (figure 2.3). This change leads to a situation where the timing of fungicide application has an effect on selection for resistance as expected (van den Berg *et al.*, 2013, 2016) but is no longer so powerful that it dominates all other effects.

Moving the start of the season also required altering the values of certain parameters of the model. Where possible we used values from van den Berg *et al.* (2013), which represents the most similar model in the literature to the original models in Hobbelen *et al.* (2011b). This model also includes five leaf layers. However, we chose to refit the infection rate parameter (β) as there is no reason for it to have the same value between outwardly-similar models. We fit the parameter by using least squares minimisation between the amount of infectious tissue in the original Hobbelen model and in the model starting two phyllochrons earlier. The minimisation was carried out on daily amount of infectious tissue from GS32 onward.

Critique

Degree-days are used in this model to account for the effect of temperature on the crop and pathogen. However this is somewhat underused in the model as the mean daily temperature is assumed to be constant. In addition the use of this measure of time throughout the model implicitly assumes that pathogen, crop and fungicide dynamics all scale in the same way with temperature, which is unlikely. Fortunately for our purposes this is unimportant, as the model parameters have been fit with this in mind and we are uninterested in the effects of temperature.

It is assumed in this model that protectant and eradicant effects have the same dose-

response curve parameters. This is very unlikely to be the case, even if fungicides show the same degree of protectant and eradicant activity in the field (see section 7.5.2 for more discussion of this point).

2.4.3 ElevenLeaf model

Model description

We also use a more complex model for septoria, which is based on the models introduced by van den Berg *et al.* (2013) and Kitchen *et al.* (2016). This model explicitly tracks 11 layers of wheat leaf, and uses a very similar structure to the FiveLeaf model.

The way in which the different leaf layers are modelled is significantly different to the FiveLeaf model. Instead of amalgamating the layers into a single state variable, the infection status of each leaf is tracked separately. This allows tracking the differential interception of fungicide by each leaf layer, due to size, and also accounts for some of the effects of the spatial arrangement of leaves on epidemic progress. The life-cycle of each leaf is modelled as emergence, extension, senescence and death. Each leaf first appears at its emergence time ($T_{\text{EMERGE, i}}$) and begins growing at a rate determined by the leaf-specific carrying capacity. After a time, if the leaf is one of the topmost 4 leaves, extension begins at T_{EXTEND} and the leaf begins to move up the stem from the point at where it emerged; all leaves emerge at the height of the previous leaf. The leaf extends to a maximum internode distance of 10cm at constant speed over the course of 100 degree-days. Growth stops at the same time that senescence begins (at $T_{\text{SENES, i}}$). Note that senescence takes a different mathematical form to the FiveLeaf model. The change in the total area of a single leaf is given by

$$\frac{\mathrm{d}A_i}{\mathrm{d}t} = g_i(t) \tag{2.25}$$

$$= \begin{cases} r(k_i - A_i), & \text{for } T_{\text{EMERGE},i} \leq t < t_{\text{SENES},i} \\ 0, & \text{otherwise} \end{cases}, \tag{2.26}$$

and the senescence rate for living tissue is,

$$\Gamma_i(t) = \begin{cases} \mathrm{e}^{0.05(t - T_{\text{DEATH},i})}, & \text{for } T_{\text{SENES},i} \leq t < T_{\text{DEATH},i} \\ 0, & \text{otherwise} \end{cases}. \tag{2.27}$$

At the time of death the size of the leaf is set to zero.

As with the FiveLeaf model, fungicide applications are modelled as discrete changes. However the amount of fungicide that each leaf intercepts depends on its size and how many leaves are emerged and above it. The amount of fungicide intercepted by each leaf layer increases with its area and decreases with the product of the areas of the leaves above. The

dose intercepted by a single leaf (D_i) is related to the dose sprayed (D_0) at a time t is given by

$$p(i,t) = \mathrm{e}^{-\tau A_i} \tag{2.28}$$

$$D_i(t) = D_0\Big(1 - p(i,t)\Big)\left(\prod_{j=1}^{i-1} p(j,t)\right), \tag{2.29}$$

where τ is the proportional projection of the leaf surface onto a horizontal plane. This dose is then diluted over the volume of the leaf and decays over time to give the fungicide concentration,

$$C_i(t) = \frac{D_i(T_{\text{SPRAY}})}{qA_i}\mathrm{e}^{-\delta(t-T_{\text{SPRAY}})}, \tag{2.30}$$

where q is the thickness of the leaf and δ is the fungicide exponential decay rate. Note that the fungicide concentration decreases over time due to both decay of the chemical and growth of the leaf. The model as presented in Kitchen *et al.* (2016) also includes seed treatments and associated dynamics such as fungicide movement through the xylem, which we remove for simplicity.

As in the FiveLeaf model infection is assumed to obey frequency-dependent transmission, however now the rate of secondary infection depends on the distance between the infectious and healthy tissue. Secondary infection occurs due to splash dispersal of pycnidiospores, and so the rate of transmission from a lower to higher leaf is smaller than the rate of transmission in the opposite direction. The scaling for the rate of transmission from leaf i to j is given by

$$z(i,j) = \begin{cases} 1, & \text{for } i = j \\ \mathrm{e}^{-b_{\text{DOWN}}D(i,j,t)}, & \text{for } i > j \\ \mathrm{e}^{-b_{\text{UP}}D(i,j,t)}, & \text{for } i < j \end{cases}, \tag{2.31}$$

where $D(i,j,t)$ is the distance between leaves i and j at time t. The parameters b_{DOWN} and b_{UP} set the relative rates of upward and downward dispersal.

A key change compared to the FiveLeaf model is the addition of multiple latent (E) compartments. This is done for two main reasons. Firstly, this generates a more realistic situation where the rate of becoming infectious depends on how long the tissue has been infected (Cunniffe *et al.*, 2012; van den Berg *et al.*, 2013). Secondly, field experiments have shown that eradicant fungicides tend to only be effective in the earlier part of the latent period of infection (van den Berg *et al.*, 2013), and this formulation allows the restriction of fungicide action to only the first $\frac{n}{2}$ of n latent compartments. The model uses 10 latent compartments.

The earlier version of the model (van den Berg *et al.*, 2013, 2016) only explicitly models the epidemic and fungicide on the first five leaf layers but we follow the later version (Kitchen

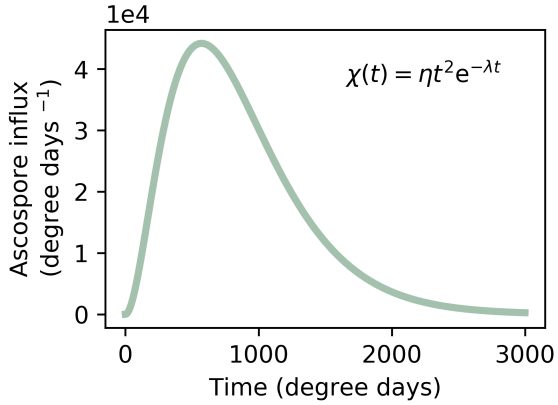

Figure 2.4: The influx of ascospores against time in the ElevenLeaf model. Ascospores are the source of primary infection. Parameter values are given in appendix A.1.3.

et al., 2016) in modelling 11 layers explicitly, and modelling primary infection as a time-varying rain of ascospores affecting all leaf layers rather than infection from lower leaves. The infectious pressure from fungicide-resistant and -sensitive ascospores is modelled as (figure 2.4)

$$\chi(t) = \eta t^2 e^{-\lambda t} \tag{2.32}$$

$$P_R(t) = \phi\chi(t) \tag{2.33}$$

$$P_R(t) = (1-\phi)\chi(t)\,, \tag{2.34}$$

where ϕ is the resistance frequency of ascospores. As with the FiveLeaf model this is updated at the end of each season to match the resistance frequency of the infectious compartments. Only the top 5 leaves contribute to determining the resistance frequency in the next season; the resistance frequency in the next season is given by

$$\phi = \frac{\sum_{i=1}^{5} I_{R,i}(T_{DEATH,i})}{\sum_{i=1}^{5} I_{R,i}(T_{DEATH,i}) + \sum_{i=1}^{5} I_{S,i}(T_{DEATH,i})}\,. \tag{2.35}$$

The following system of ODEs describes the dynamics of the ith leaf layer (figure 2.5),

$$A_i = S_i + \sum_{j=1}^{10}(E_{R,i,j} + E_{S,i,j}) + I_{S,i} + I_{R,i} + R_i \tag{2.36}$$

$$\frac{\mathrm{d}S_i}{\mathrm{d}t} = g_t(t) - \Gamma_i(t)S_i \tag{2.37}$$

$$- \frac{S_i}{A_i}\Bigg(\beta_I\Big(1-\epsilon_L(C_L)\Big)\sum_{j=1}^{11} z(i,j)\left(\Big(1-\epsilon_H(C_H)\Big)I_{S,j} + I_{R,j}\right) \tag{2.38}$$

$$+ \beta_P\Big(1-\epsilon_L(C_L)\Big)\Big(P_R + P_S\Big(1-\epsilon_H(C_H)\Big)\Big)\Bigg) \tag{2.39}$$

$$\frac{\mathrm{d}E_{R,i,j}}{\mathrm{d}t} = \begin{cases} \frac{S_i}{A_i}\Big(1-\epsilon_L(C_L)\Big)(\beta_I \sum_{k=1}^{11} z(i,j) I_{R,k} + \beta_P P_R) & \\ \quad -(\Gamma_i(t)+\gamma)E_{R,i,j}, & \text{for } j=1 \\ \gamma(E_{R,i,j-1} - E_{R,i,j}) - \Gamma_i(t)E_{R,i,j}, & \text{for } j>1 \end{cases} \tag{2.40}$$

$$\frac{\mathrm{d}E_{S,i,j}}{\mathrm{d}t} = \begin{cases} \frac{S_i}{A_i}\Big(1-\epsilon_H(C_H)\Big)\Bigg(\Big(1-\epsilon_L(C_L)\Big) & \\ \quad \Big(\beta_I \sum_{k=1}^{11} z(i,j) I_{S,k} + \beta_P P_S\Big) - \gamma E_{S,i,j}\Bigg) & \\ \quad -\Gamma_i(t)E_{S,i,j}, & \text{for } j=1 \\ \gamma\Big(1-\epsilon_H(C_H)\Big)(E_{S,i,j-1} - E_{S,i,j}) - \Gamma_i(t)E_{S,i,j}, & \text{for } 1<j<6 \\ \gamma\left(\Big(1-\epsilon_H(C_H)\Big)E_{S,i,j-1} - E_{S,i,j}\right) - \Gamma_i(t)E_{S,i,j}, & \text{for } j=6 \\ \gamma(E_{S,i,j-1} - E_{S,i,j}) - \Gamma_i(t)E_{S,i,j}, & \text{for } j>6 \end{cases} \tag{2.41}$$

$$\frac{\mathrm{d}I_{R,i}}{\mathrm{d}t} = \gamma E_{R,i,10} - \mu I_{R,i} \tag{2.42}$$

$$\frac{\mathrm{d}I_{S,i}}{\mathrm{d}t} = \gamma E_{S,i,10} - \mu I_{S,i} \tag{2.43}$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \mu(I_{R,i} + I_{S,i}) + \Gamma_i(t)\left(S_i + \sum_{j=1}^{10} E_{R,i,j} + E_{S,i,j}\right) . \tag{2.44}$$

Note that the infection rate parameters for primary (β_P) and secondary (β_I) infection are now different, whereas they had the same value in the FiveLeaf model. Also note that the fungicide-resistant and -sensitive latent compartments show different dynamics because only the high-risk fungicide is modelled as having eradicant action.

Yield is measured similarly to the FiveLeaf model, taking the integral of photosynthetically-active tissue between GS61 and GS87 across the top 3 leaves,

$$\text{Yield} = \int_{T_{GS61}}^{T_{GS87}} \sum_{i=1}^{11}\left(S_i + \sum_{j=1}^{10}(E_{S,i,j} + E_{R,i,j})\right)\,\mathrm{d}t\,. \tag{2.45}$$

Fitting

The paper introducing this model presented a set of parameter values for a generic SDHI fungicide on typical septoria epidemic in the UK. Unfortunately we discovered a software bug in the implementation of the model used in the paper, which leads to a miscalculation of the effect of the fungicide. This bug does not affect any part of the model apart from the effect of the fungicide. In addition the wrong trigonometric function was used when calculating the relationship between the leaf angle and the interception of fungicide by a leaf, although this makes a negligible difference for the leaf angle used. Due to these errors and also to allow closer comparison to the FiveLeaf model we opted to refit the fungicide parameters. We used the same sources of data used in Hobbelen *et al.* (2011a) to fit the fungicide parameters to

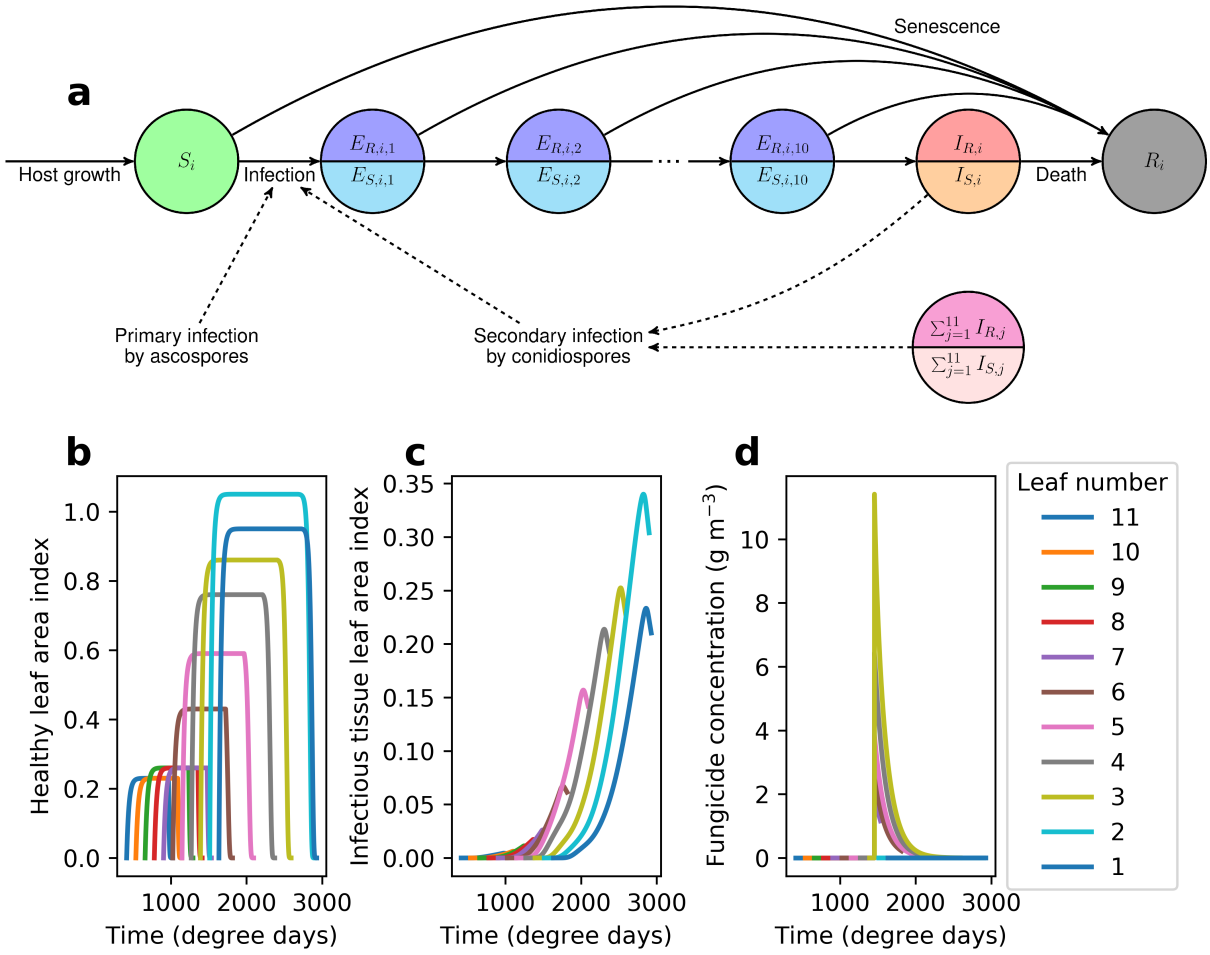

Figure 2.5: The structure and dynamics of the ElevenLeaf model. **a)** The structure of the compartmental model for a single leaf layer. Circles represent epidemiological compartments, solid lines transitions and dashed lines effects on the rates of transition. **b)** The healthy area of each leaf layer over time in the absence of disease. **c)** The amount of infectious tissue on each leaf layer over time when no fungicide is applied. **d)** The amount of fungicide on each leaf layer over time for a single spray at GS32. Dynamics are shown for a single season using default parameter values (appendix A.1.3).

represent chlorothalonil and pyraclostrobin. Apart from the parameters that were refit, all other parameter values used are as in Kitchen *et al.* (2016).

The data used for fitting was the disease severity measured at two time points in the season, after a single spray of fungicide was applied at a range of doses (Lockley & Clark, 2005; Paveley *et al.*, 1998). The level of disease without fungicide was significantly lower in the ElevenLeaf model compared to the data, and so we normalised the severity data by the untreated severity. We then used the L-BFGS-B optimisation algorithm to minimise the squared differences between the observed relative severities and those produced by the model, for matching application times and doses.

It was notable that the best-fitting fungicide parameters did not lead to relative disease

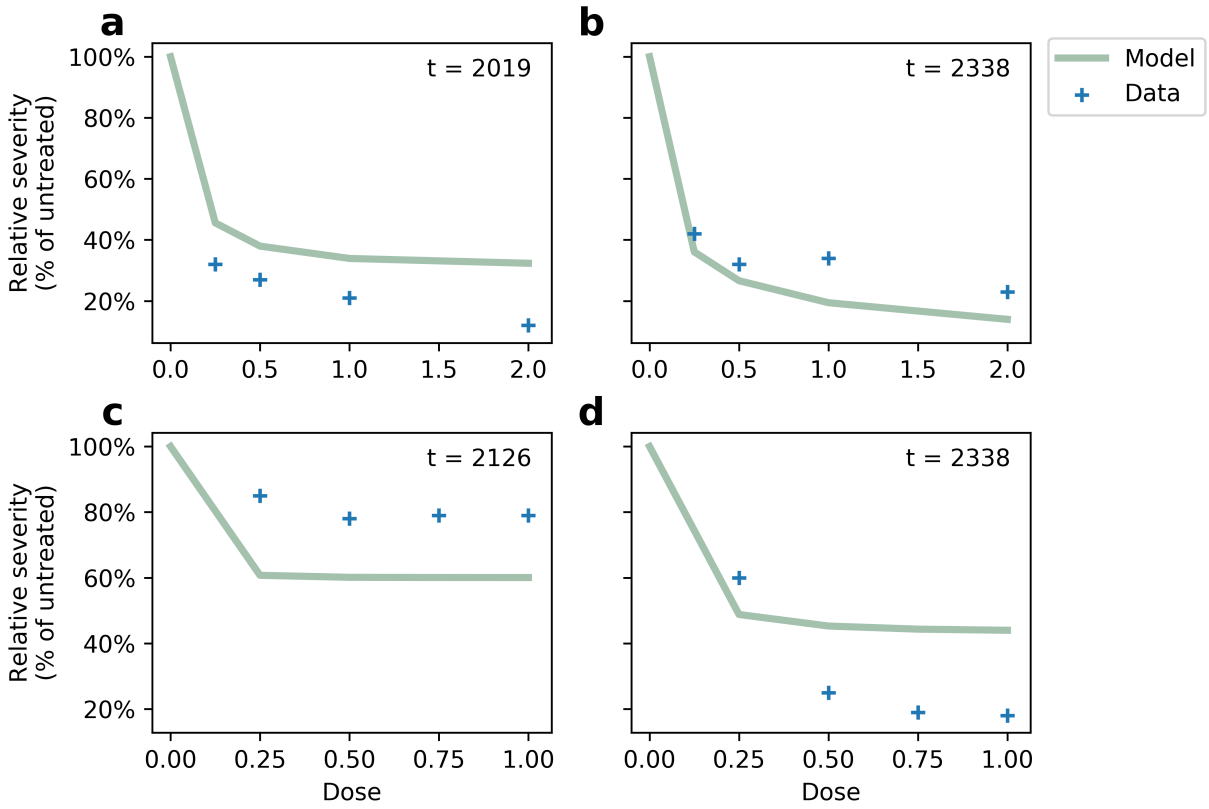

Figure 2.6: The output of the ElevenLeaf model for the best-fitting fungicide parameters compared to the data used for fitting. The data and model output are shown for **a & b)** pyraclostrobin and **c & d)** chlorothalonil. **a & c)** show the comparison for the earlier observation time and **b & d)** the later in each case.

severities that particularly closely matched those of the data (figure 2.6). However this is likely a shortcoming of the model rather than the fitting technique. For each fungicide, to better fit the data at one time point requires the fungicide to be more efficacious and at the other to be less efficacious. The fit might be improved by allowing the dose-response curves to vary for protectant and eradicant effects, but the available data was already limiting and the effect of such model refinements was decided to be outside the scope of our work.

Critique

Firstly, this model shares the same issue with the use of degree-days as the FiveLeaf model; the daily temperature is assumed constant and pathogen, fungicide and host dynamics are all assumed to scale in the same way with the number of degree-days accumulated. However, the earlier start time of this model does avoid some of the issues with edge effects that occur with fungicide applications near the beginning of the season. As with the FiveLeaf model, the assumption that protectant and eradicant dose-response curves are the same is unlikely to be realistic.

In this model the area of each leaf is modelled separately, and this area has multiple effects. For example, the amount of fungicide intercepted by a given leaf area is proportional

to the area of the leaf. The area of any leaf is monotonically increasing until it is removed from the simulation at its time of death. This seems unlikely to represent biological reality, but we do not have sufficient data to validate or improve this feature.

There are a pair of notable oversights in the function giving the infection probability between different leaf layers. Firstly it does not take into account the presence of intermediate leaves. That is, the probability of a spore landing on a given receptor leaf from a specific donor leaf is dependent only on the distance between them and which one is higher up the stem. Clearly the area of the leaf layers between that pair of leaves is likely to impact on the probability of a spore landing on the receptor leaf, rather than a leaf in-between. Secondly the lower leaves (below leaf 4) do not extend up the stem and are instead considered to remain at the same height. However the infection probability is still weighted to be more likely from a leaf that emerged later rather than one earlier. This is motivated by the mechanics of splash dispersal for leaves that are at different heights, but it is much harder to justify for leaves at the same height. Each of these cases are partially accommodated for by the fact that the infection rate parameter is fit to data.

The contribution of each of the top five leaves toward resistance in the next season is decided at the time of their death. Consequently the relative contribution of each leaf is determined by its size just before death. This varies between leaves in a relatively arbitrary way and may affect the results. However it is not clear whether alternative choices such as later leaves contributing more or all leaves contributing equally are necessarily better.

2.5 Powdery mildew of French grapevine

2.5.1 The pathosystem

Grapevine may not constitute a key source of calories or nutrients on a global scale, but is an economically important crop in many countries for both fruit and wine production. The vast majority of grapevine grown is of the European species *Vitis vinifera*, which is highly susceptible to powdery mildew caused by *Erysiphe necator*. *E. necator* represents a relatively recent invader in Europe, only arriving from its native range in North America in the nineteenth century (Gadoury *et al.*, 2012). The lack of historical co-evolution between the pathogen and host in Europe means that there is very little natural powdery mildew resistance in *V. vinifera*; the same is true of the other most important grapevine disease downy mildew (*Plasmopara viticola*) (Calonnec *et al.*, 2013). The lack of host resistance means that control of powdery mildew in Europe relies on intense fungicide application regimes. North American species of grape or hybrids can show reasonable resistance but are generally not an economically viable replacement as the produced wine is considered to be of lower quality (Gadoury *et al.*, 2012).

We introduce a model of this pathosystem here to provide a test of the generality of the

results generated in our work with the septoria pathosystem.

2.5.2 The model

This model is based on the models used in Burie *et al.* (2011) and Mammeri *et al.* (2014). These are ordinary differential equation models which have been parameterised to fit a more complicated 3D simulation of powdery mildew on a single grapevine (Calonnec *et al.*, 2008). Mammeri *et al.* (2014) embeds this model in a reaction-diffusion model, but we only use the parts relevant to a single vine. There is therefore an implicit assumption, also present in both of the models of septoria, that the dynamics on a single plant extend to the rest of the crop.

In the same way as the models of septoria, the core of this model is a classic SEIR model (figure 2.7). The main deviation from this is the addition of a compartment for tissue which has developed ontogenic resistance (O). This accounts for the fact that both leaf and berry tissue become increasingly resistant to powdery mildew infection as they age. This is represented in the model by having a constant flow of susceptible tissue into the ontogenically-resistant class.

The unit of time used in the model is the calendar day and the unit of the tissue state variables is area of leaf in cm^2. The total area of tissue grows logistically,

$$\frac{\mathrm{d}A}{\mathrm{d}t} = r(t)A\left(1 - \frac{A}{k(t)}\right) . \tag{2.46}$$

The two parameters of the growth rate (r and k) are time-dependent in this model due to the agronomic practice of shoot topping. Shoot topping refers to the typical practice in vineyards of cutting back the top of the shoots some time after flowering to encourage the growth of secondary shoots. Shoot topping was included in both Burie *et al.* (2011) and Mammeri *et al.* (2014) but modelled slightly differently in these two papers. In both papers shoot topping is modelled by reducing the size of each epidemiological compartment by a particular percentage and in addition changing the value of some model parameters (in our notation r, k, β). In Burie *et al.* (2011) this percentage is the same for all compartments whilst in Mammeri *et al.* (2014) the percentage varies between compartments. The motivation behind a different percentage being removed from each class was that the 3D model, from which the ODE system was originally developed, showed that the infection status of the grapevine varied with height and shoot topping only removes the very upper shoots. However, the exact percentage removed from each compartment thus depends on the progression of disease, and since we alter that progression with fungicide applications, we opted for the simpler assumption that the percentage removed from each class is equal. The original percentage used in Burie *et al.* (2011) was not quoted in the paper but was calculated from the information provided on the area of leaf tissue before shoot topping ($A(T_{TOP})$) and the area of leaf tissue at day 211 ($A(211)$). By solving the logistic growth equations and

substituting these values and the host growth parameter values,

$$\begin{aligned} x &= 1 + \frac{A(211)k}{A(211)A(T_{TOP})(\mathrm{e}^{rt} - 1) - kA(T_{TOP})\mathrm{e}^{rt}} \\ &\approx 0.2\,, \end{aligned} \tag{2.47}$$

where x is the proportion of leaf area lost to shoot topping. The infection rate obeys frequency dependent transmission and only a single latent compartment is included.

The earlier papers do not consider fungicide, and the later paper (Mammeri *et al.*, 2014) has only a very simple treatment of fungicide, and so we opt to utilise the model of fungicides first introduced in Hobbelen *et al.* (2011b). Fungicides are generally applied many more times and with a greater variety of active ingredients to grapevine over a single season than to wheat. However, for our work we are only interested in the interaction of a single low-risk and high-risk fungicide. Experimental studies have begun to show that applying just two sprays of fungicide during the month after fruit set can provide adequate control as the berries rapidly become resistant to infection (Gadoury *et al.*, 2003). Despite common agronomic practice generally using a higher number of sprays (see above), we model only two applications of fungicide, two days before flowering and another fourteen days later. The system of ODEs are then

$$A = S + E_R + E_S + I_R + I_S + R + O \tag{2.48}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = r(t)A\left(1 - \frac{A}{k(t)}\right) - \beta(t)\frac{S}{A}\big(1 - \epsilon_L(C_L)\big)\Big(I_R + \big(1 - \epsilon_H(C_H)\big)I_S\Big) - mS \tag{2.49}$$

$$\frac{\mathrm{d}E_R}{\mathrm{d}t} = \beta(t)\frac{S}{A}\big(1 - \epsilon_L(C_L)\big)I_R - \big(1 - \epsilon_L(C_L)\big)\gamma E_R \tag{2.50}$$

$$\frac{\mathrm{d}E_S}{\mathrm{d}t} = \beta(t)\frac{S}{A}\big(1 - \epsilon_L(C_L)\big)\big(1 - \epsilon_H(C_H)\big)I_S - \big(1 - \epsilon_L(C_L)\big)\big(1 - \epsilon_H(C_H)\big)\gamma E_S \tag{2.51}$$

$$\frac{\mathrm{d}I_R}{\mathrm{d}t} = \big(1 - \epsilon_L(C_L)\big)\gamma E_R - \mu I_R \tag{2.52}$$

$$\frac{\mathrm{d}I_S}{\mathrm{d}t} = \big(1 - \epsilon_L(C_L)\big)\big(1 - \epsilon_H(C_H)\big)\gamma E_S - \mu I_S \tag{2.53}$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \mu(I_R + I_S) \tag{2.54}$$

$$\frac{\mathrm{d}O}{\mathrm{d}t} = mS \tag{2.55}$$

$$\frac{\mathrm{d}C_H}{\mathrm{d}t} = -\delta_H C_H \tag{2.56}$$

$$\frac{\mathrm{d}C_L}{\mathrm{d}t} = -\delta_L C_L\,. \tag{2.57}$$

Note that unlike the models of septoria, both the high-risk and low-risk fungicide are assumed to have both protectant and eradicant action.

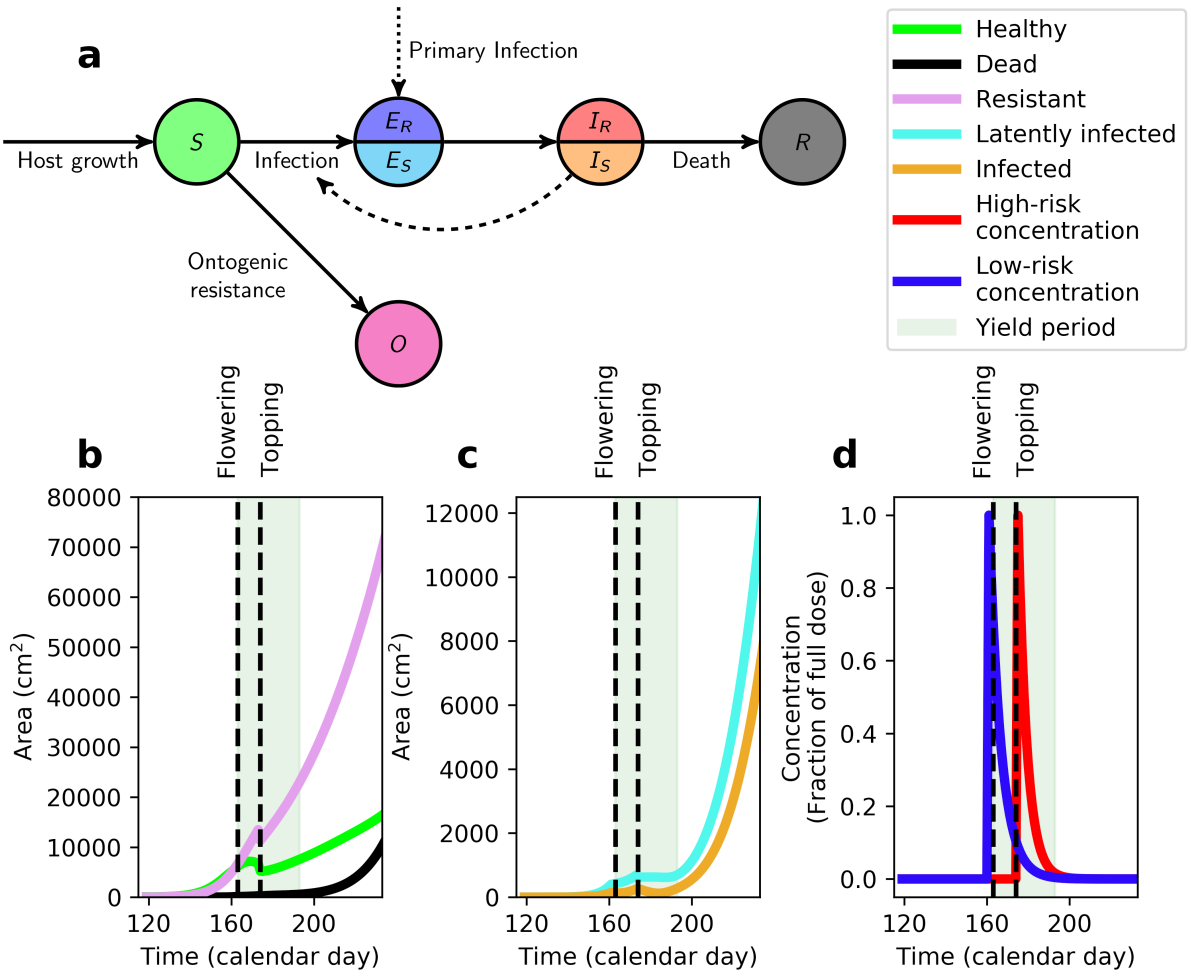

Figure 2.7: The structure and dynamics of the powdery mildew model. **a)** A schematic of the model structure. Circles are epidemiological compartments (split into two where necessary for each pathogen strain), solid lines are transitions, dashed lines represent effects on the rates of transitions and the dotted line shows the point of initial infection. **b - d)** The dynamics of the model when a full dose of the low-risk is applied at the first spray and of the high-risk at the second. **b)** The amount of healthy and dead tissue over time. **c)** The amount of infected tissue over time. **d)** The amount of fungicide over time. Dynamics are shown for a single season using the default parameters (appendix A.2).

2.5.3 Parameterisation

As the original models did not include the effect of fungicide, we had to fit the fungicide parameter values. We chose trifloxystrobin as our high-risk fungicide and sulphur as our low-risk. Typically sulphur would be applied very early and very late in the season rather than the timings we use, but we are more interested in understanding fungicide resistance dynamics rather than recreating exact management tactics. The model and analysis could be readily re-run for the individual agronomic practices of particular vineyards.

Firstly we use the literature to find values for the half-life of trifloxystrobin on grapevines as 3 days (Jyot *et al.*, 2010). We could not find data for the persistence of sulphur on grapevine

but it has a half-life of around 4 days on other crops (Nasr, 2010). It was notable that these studies identified persistence that did not match up with exponential decay; generally the rate of decay was more rapid when there was more residue remaining. However, a previous paper using similar models investigated the effect of switching out exponential decay for a gamma distribution and found no effect on the qualitative results (Hobbelen *et al.*, 2014). Therefore we continue to use the simpler exponential model.

The exponential dose-response curves that we use require at an absolute minimum effectiveness data at two doses to be fit, and Michaelis-Menten responses are the same. However, it proved surprisingly difficult to find experimental data in the literature that measured effectiveness at more than one dose for any grapevine fungicide. Instead, we assume that the asymptotic maximum effect (ω) of the fungicides is 1. This represents the assumption that given sufficiently large quantities of fungicide the growth of the pathogen is almost entirely suppressed. This allows us to fit the remaining dose-response parameter to data with only a single dose of fungicide. Reuveni (2001) gives the reduction in the severity of powdery mildew on berries when applying 6 sprays of sulphur or of trifloxystrobin. Although our model tracks leaf infection rather than berry, we assume that the effect of fungicide on epidemic sizes on both is closely related. Therefore we use least-squares minimisation to choose a value for the curvature parameters such that the reduction in disease severity on the leaves compared to untreated is the same as the available data.

There is no consideration of the effect of powdery mildew infection on yield or grape quality in the papers introducing the pathosystem model. Of course, it is desirable to be able to output such a metric from the model as this is the main concern of growers with regards to infection. When implementing a simple estimate of damage due to infection, we worked on the assumption of the grapevine being cultivated for wine production. Ultimately, infection on the berries is the cause of reduction in quantity and quality of wine produced, but this relationship is complex and poorly understood (Calonnec *et al.*, 2004; Pool *et al.*, 1984). The model does not track the infection status of berries but only leaf tissue. However, there is a strong positive correlation between the extent of powdery mildew infection on grapevine leaves and berries (Calonnec *et al.*, 2006; Delière *et al.*, 2015). Given the complexities involved in producing an accurate model for yield prediction and the many other uncertainties present in the model, we opt for a simple model of yield. Berries become highly resistant to powdery mildew infection within a month of flowering (Gadoury *et al.*, 2003), and so we designate the time period between flowering and 30 days later as the critical period for yield formation. The maximum infection severity within this time is,

$$\text{Infection severity} = \sup_{163 \leq t \leq 193} \left(\frac{I_R(t) + I_S(t)}{A(t)} \right) . \tag{2.58}$$

We then assume that if the severity of disease increases above a particular threshold, disease control was unacceptably poor and the yield or quality of wine produced is uneconomical.

This is very similar to the way in which yield is treated in the septoria models. This threshold value for infection was chosen to match reality such that control was possible at mid to high concentrations of fungicide, control was unacceptable at low doses of both fungicides, and sufficient control by the low-risk alone was impossible. The final value that was chosen was 3%, which matches closely with the stringent requirements for disease control in France; grower tolerance for berry infection incidence ranges between 0 - 3% (Deliere *et al.*, 2010). If anything, this threshold value may be a little high as the berries generally show the same or greater amount of infection compared to the leaves (Calonnec *et al.*, 2006).

2.6 Notable exclusions and simplifications from all models

Each model makes further simplifying assumptions which we will make explicit here.

2.6.1 Mutation

None of the models consider the generation of *de novo* resistance via mutation. This is mainly due to the fact that we use the models for investigating the spread phase of resistance (see section 1.2). Mutation would be a key process when investigating the emergence phase of resistance, but during the spread phase the changes in resistance due to population dynamics act on a much faster timescale than any changes due to mutation. For example mutation rates for novel resistance on the scale of 10^{-5} to 10^{-9} per generation have been measured, depending on fungal species and fungicide (Leonard & Fry, 1989). Selection alone however can increase the frequency of resistance in a field from around 5% to near fixation over a single season (Fraaije *et al.*, 2002). In addition, the effect of different mutation rates can to an extent be emulated by changing the initial frequency of resistance.

Modelling mutation explicitly might be more important when considering the case of resistance to a fungicide with multiple target sites or resistance to multiple fungicides, where the frequency of rare strains with multiple resistance mutations are more important (Hobbelen *et al.*, 2014; Mikaberidze *et al.*, 2017).

2.6.2 Fitness costs

In each model, it is implicitly assumed that there are no costs to resistance as in the absence of fungicides both strains have the same fitness. This modelling decision was made for two main reasons. Firstly, it is not clear what the typical magnitude of costs is or even if they exist in general (see 1.3). Secondly, we present our results as a pessimistic, but possibly realistic, scenario where resistance is inevitable.

2.6.3 Sexual reproduction

In each model we have assumed that the pathogens only reproduce asexually, despite the fact that in reality the modelled pathogens carry out both asexual and sexual reproduction (Brewer *et al.*, 2012; Suffert *et al.*, 2010). This is very typical for the fungicide resistance modelling literature although models considering sexual reproduction of plant fungal pathogens do exist (Eriksen *et al.*, 2001; Shaw, 1989a). Only considering asexual reproduction simplifies the models greatly however, and for both pathogens asexual reproduction is dominant during the season (Cortesi *et al.*, 2004; Suffert *et al.*, 2010).

2.7 Metrics

Each of these models is used to compare the performance of different fungicide application tactics, and so clearly we need some metrics to define how good a given tactic is. A number of different metrics for fungicide tactic performance have been defined in the literature previously, and are neatly summarised in van den Bosch & Gilligan (2008). All metrics consider aspects of resistance build-up, yield production or both. Historically there has been a focus on resistance build-up alone (*e.g.* Gubbins & Gilligan (1999); Kable & Jeffery (1980); Parnell *et al.* (2005); Skylakakis (1981)) but with a more recent shift toward the inclusion of yield- or control-based metrics (*e.g.* Hobbelen *et al.* (2013); Shaw (2000); van den Berg *et al.* (2013, 2016)). This is partly because only in the more modern literature have the models been complex enough to extract accurate predictions of yield, although predictions of control pre-date these yield-predicting models. The desire to use models to inform fungicide policy naturally requires a consideration of disease control, as this is the key driver for the use of fungicides in the first place.

The most common resistance-based metrics are takeover time and the selection ratio. Takeover time (T^*) is the time taken for the resistance frequency to hit a certain threshold (χ), starting from the first time the fungicide is used. The selection ratio (S_R) is the proportional increase in resistance frequency over a season, normally the first. More formally if ϕ_t is the resistance frequency at the start of season t, then

$$T^* = \inf\left\{ t \middle| \phi \geq \chi \right\} \tag{2.59}$$

$$S_R = \frac{\phi_1}{\phi_0}\,. \tag{2.60}$$

The main advantages of takeover time are that it is more intuitive to understand and is more closely related to commonly measured experimental variables (van den Bosch & Gilligan, 2008).

The selection ratio is less related to experimental data but allows faster computation (only one season must be simulated) and gives similar results to takeover time. The choice

of the threshold value for takeover is relatively arbitrary as it does not map directly onto any particular outcome of resistance development (*e.g.* failure of control). In our work we therefore prefer the selection ratio as the measure of the strength of selection for resistance.

The relationship between the selection ratio and the efficacy of a tactic in practice is not simple. Therefore we use metrics that account for the level of disease control to attempt to estimate the efficacy of tactics in the field. The most common metric for assessing the impact of fungicide resistance on yield is the effective life. This is defined as the time from first application of the fungicide until control breaks down to the extent that a critical threshold of yield loss due to disease is reached. This however does not account for the fact that tactics which produce similar effective lives may have significantly different levels of disease control over that lifetime. Therefore in the models of septoria we prefer a metric similar to the additional green leaf area duration used in Hall *et al.* (2007), which we term the lifetime yield. We define the lifetime yield as the sum of yields over the effective life of the fungicide. As we do not explicitly calculate yield, but instead use infection severity as a proxy, in the powdery mildew model we use the effective lifetime rather than the lifetime yield as the long-term yield metric in that case.

Chapter 2 Summary

- Three main models of fungicide resistance evolution are used throughout this thesis.
- Each model tracks a fungicide-resistant and -sensitive strain, considering only asexual reproduction and ignoring mutation.
- Two models of septoria leaf blotch on UK winter wheat, a simpler referred to as the FiveLeaf model and a more complex ElevenLeaf model.
- The third main model is based on powdery mildew of grapevine.
- These models allows us to simulate the response of pathogen populations and yield to particular fungicide application tactics.

3

Is mixture a better fungicide resistance management tactic than alternation?

> *If you strike me down, I shall become more powerful than you can possibly imagine.*
>
> Obi-Wan Kenobi, *Star Wars Episode IV: A New Hope*, 1977

3.1 Introduction

As introduced in section 1.8, mixture and alternation are two different tactics for managing fungicide resistance. In brief, mixture refers to applying two or more fungicides at once, whilst alternation refers to applying those fungicides sequentially. Alternation reduces selection for resistance by reducing the time of exposure to fungicide compared to spraying the high-risk fungicide at every spray. Mixture reduces selection for resistance by suppressing the growth rate of resistant pathogen strains during the period within which the high-risk is applied. Under either tactic the dose of the high-risk may be able to be reduced as the low-risk contributes to disease control, further reducing selection.

The question of whether mixture or alternation is superior for resistance management is an old one in terms of the fungicide resistance literature. The first modelling papers addressing this issue were published in the early 1980's (Delp, 1980; Kable & Jeffery, 1980), but even in a relatively recent workshop this question of which is better was identified as an unresolved problem (Zlof & Sunley, 2011). There are a few reasons why this question may be proving so difficult to resolve.

- **Difficulties in reconciling theoretical and modelling studies** Theoretical and modelling studies are very useful tools for understanding biological systems, and for generating new hypotheses. Ultimately however, they must be tested against lab and field experiments to make sure that the necessary simplifying assumptions have not invalidated the results. It is intrinsically difficult to verify modelling studies on fungicide resistance. For example: lab and field results do not necessarily agree, large time and monetary costs are associated with field trials, variability between sites and years can have large effects on field trial results, fungicide resistance is often present at a frequency where it is difficult to detect or get accurate measurements, and fungicide

resistance modelling studies are generally concerned with too long a time-scale to be feasibly verified experimentally.

- **The timescales involved in fungicide resolution evolution** The effects of fungicide resistance can take a long time to manifest, and so individual field trials are unlikely to be sufficient. Within an area, growers likely use a variety of fungicide application methods; this combined with the long distance dispersal of many fungal pathogens makes relating data on historical fungicide use to resistance development difficult.

- **Confounding factors** There are also a number of confounding factors that need to be considered when comparing alternation and mixture in a modelling study, which can have large effects on the prediction of which is better. For example, the modelling literature of the 1980's was heavily concerned with the nature of synergy between fungicides (see Shaw (1989a) for review). While an important question, resolving it does not necessarily get one any closer to deciding between mixture and alternation in practice.

The theme for the question of mixture and alternation was set by some of the very first modelling papers, with one concluding that alternation was almost always better (Kable & Jeffery, 1980) and the other mixture (Skylakakis, 1981). Since then there have been a number of experimental and modelling papers investigating this question. A recent review found that of 12 experimental cases: 6 demonstrated less selection from mixture, 2 from alternation, and the remainder no difference (van den Bosch *et al.*, 2014a). Modelling studies showed a similar trend. Although there is variability in the literature, mixture is generally seen as the superior tactic for resistance management. More recently there has been shift toward incorporating the level of disease control imposed by tactics when considering performance. Ultimately a resistance management tactic that guarantees low rates of resistance development but also low levels of control is effectively useless, since for example spraying no fungicide at all removes the risk of resistance development.

The most recent modelling works on the question of mixture and alternation are Hobbelen *et al.* (2013) and Mikaberidze *et al.* (2014). The former investigates the question of mixture and alternation of two high-risk fungicides. This work used the model upon which we based the FiveLeaf septoria model described in section 2.4.2. Mikaberidze *et al.* (2014) focussed primarily on the mixture aspect of the question, and used analysis of a simpler model to identify cases where mixture might not lead to resistance development. The second study follows on from the conclusion of the first that mixture always leads to longer or the same fungicide effective lives compared to alternation. Despite the firm conclusion of Hobbelen *et al.* (2013) that "the mixture strategy is better than or equal to alternation strategies", this depends on the interpretation of the data. The authors of that paper compared the tactics mainly in terms of which is capable of providing the longest effective life. Of course, there are other possible comparisons - such as which is better for a given cost of fungicide or which

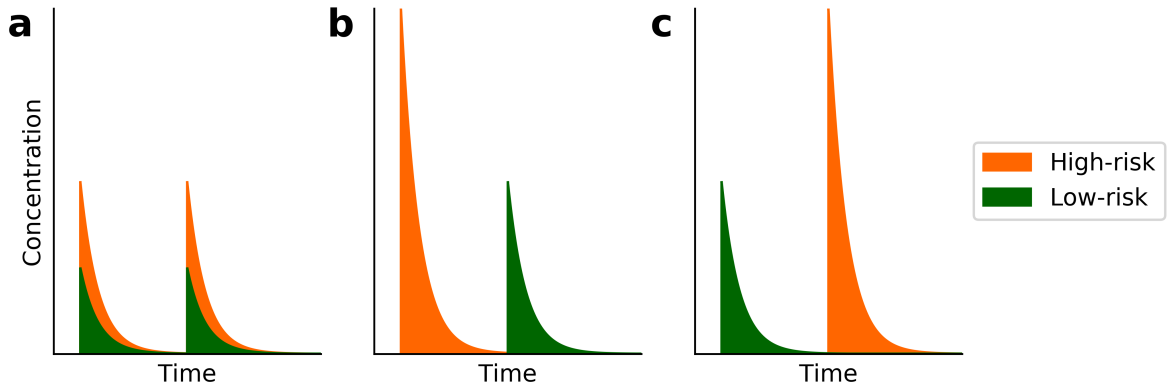

Figure 3.1: Schematics representing the fungicide application tactics modelled in this chapter. **a)** Mixture. **b)** Alternation High-Low. **c)** Alternation Low-High. Doses are halved at each application under mixture. The curved shape is due to decay of the chemicals with time.

reduces the risk of catastrophic failure most (te Beest *et al.*, 2013). The data presentation in Hobbelen *et al.* (2013) however makes it difficult to make any other comparisons or draw further conclusions.

In this chapter we aim to examine the case of mixture and alternation of a low-risk and high-risk fungicide in the FiveLeaf model of septoria. We focus on the case of a high-risk and low-risk as this remains an open question, and the most recent treatment of mixture and alternation in the literature focussed on two high-risk fungicides (Hobbelen *et al.*, 2013). We compare the tactics in a variety of ways, with comparisons designed either to aid understanding of the system or to produce predictions that can inform practice.

3.2 The tactics

For our purposes we will define 3 particular tactics for mixture and alternation of a high-risk and low-risk fungicide (figure 3.1).

1. **Mixture** Both the high-risk and low-risk are applied at every spray.
2. **Alternation High-Low** The high-risk and low-risk are alternated across sprays, with the high-risk sprayed first.
3. **Alternation Low-High** The high-risk and low-risk are alternated across sprays, with the low-risk sprayed first.

In this chapter we always consider two sprays of fungicide at the traditional key timings for septoria on UK wheat, the T1 spray at GS32 and the T2 at GS39 (Paveley *et al.*, 2014). We model azoxystrobin as the high-risk fungicide and chlorothalonil as the low-risk, using the parameterisation of the fungicide dose-response curves from Hobbelen *et al.* (2011a). The value of all parameter values used in this chapter are summarised in appendix A.1.1.

3.3 Full dose

The first comparison we will make is between the tactics at full dose. As mixture sprays each fungicide twice as often as alternation, we halve the doses under mixture to conserve the total amount of fungicide applied over the season. To compare tactics we use the selection ratio over the first season as our measure of tactic performance in terms of resistance management and the lifetime yield as our measure of long-term yield performance (see section 2.7 for definitions of these metrics). The initial frequency of the resistant pathogen strain is set to 10^{-10} and the same value is used for all later comparisons. The choice of the starting frequency is relatively arbitrary but also has little effect on the results (see section 4.2).

Each tactic leads to the same general dynamics over the seasons with a sigmoidal increase in resistance frequency to near-fixation and a sigmoidal decrease in yield to the yield provided by control by the low-risk alone (figure 3.2). We see that for the purposes of both resistance management and long-term yield the best tactic at full dose is Alternation Low-High. This tactic gives the lowest disease control in the first season, but ultimately this is out-weighed by its smaller effect on resistance development and leads to a greater lifetime yield.

Although Alternation Low-High gives the lowest initial control, its yield when the resistance trait is at fixation is actually intermediate to the two other tactics. In this situation the two alternation tactics effectively represent applying a single dose of low-risk at either T1 (Alternation Low-High) or T2 (Alternation High-Low) as the high-risk is completely ineffective. This identifies a key aspect of fungicide application timing, a spray of fungicide at T1 provides better disease control than the same dose at T2. This can also explain why Alternation Low-High initially performs worse than Alternation High-Low. The high-risk fungicide is more effective than the low-risk, and so applying it earlier in the season provides more control than the opposite alternation.

Mixture provides both a higher initial yield and a higher final yield at resistance fixation. This is due to the effect of dose-splitting. Dividing the same total dose into multiple sprays increases the total effect of fungicide, and thus control, due to the concave shape of dose-response curves. Despite providing better yields at both extremes of resistance frequency, dose-splitting carries the cost of increasing selection (by increasing the effect of the high-risk) and in this case this outweighs any benefits of mixture toward disease control.

3.4 Variable dose

Although applying both fungicides at full dose represents one typical agronomic case, there is significant evidence that reduction in the doses of fungicides can be beneficial and is done extensively in practice (Jørgensen *et al.*, 2017; van den Bosch *et al.*, 2011). Therefore we

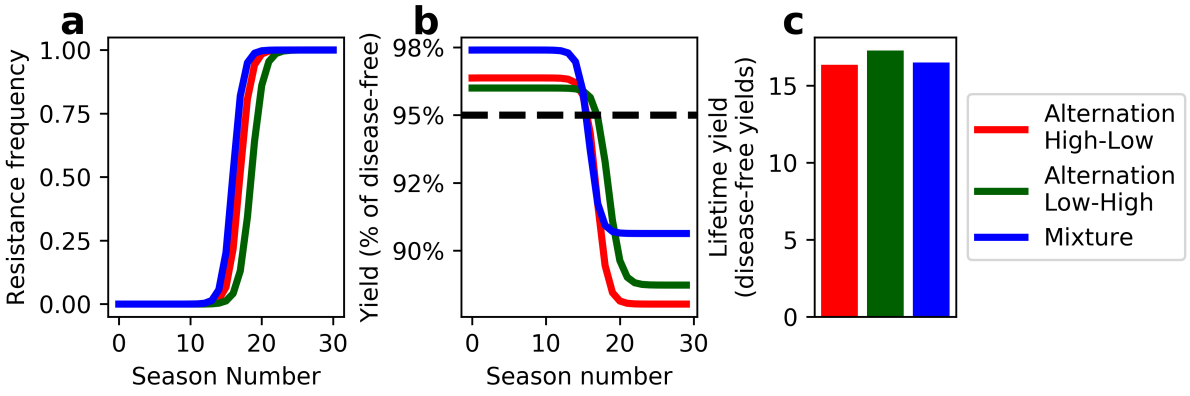

Figure 3.2: The performance of each tactic at full dose (half for mixture). **a)** The frequency of resistance at the start of each season. **b)** The yield produced each season. **c)** The overall lifetime yield produced by each tactic.

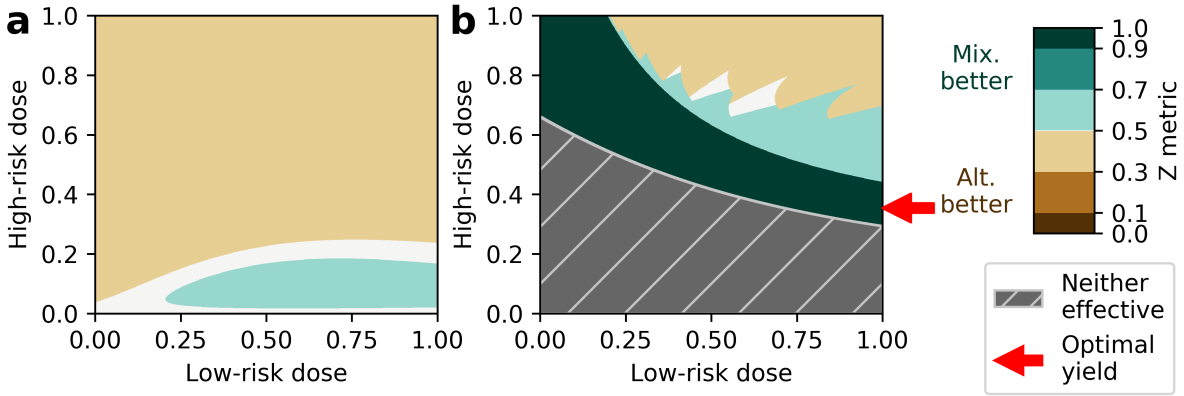

Figure 3.3: Relative performance of mixture and alternation for a variety of dose combinations. A higher value of the Z metric implies that mixture performs better and a lower alternation, a value of 0.5 means that the tactics are performing equally well. Predictions are shown based on **a)** selection ratio ($Z = \mathrm{SR_{ALT}}/(\mathrm{SR_{ALT}} + \mathrm{SR_{MIX}})$) and **b)** lifetime yield ($Z = \mathrm{LY_{MIX}}/(\mathrm{LY_{MIX}} + \mathrm{LY_{ALT}})$).

also examine the relative performance of the tactics at reduced dose, still halving doses in mixture to conserve the total dose applied.

There is a clear dependence on the doses applied as to whether mixture or alternation is superior for either resistance management or long-term yield (figure 3.3). For resistance management, mixture generally performs better at higher doses of the low-risk fungicide and alternation at higher doses of the high-risk. This makes intuitive sense as only mixture benefits from the suppressive effects of the low-risk, and the cost of dose-splitting of the high-risk increases as its dose increases. For long-term yield however we see that alternation performs better at higher doses of either fungicide and mixture at lower. There is an area of dose-space where only mixture is able to provide even one season of acceptable disease control, and so naturally out-performs alternation for long-term yield by a large margin in this space.

The patterns in dose-space can be illustrated by looking at the response to increasing

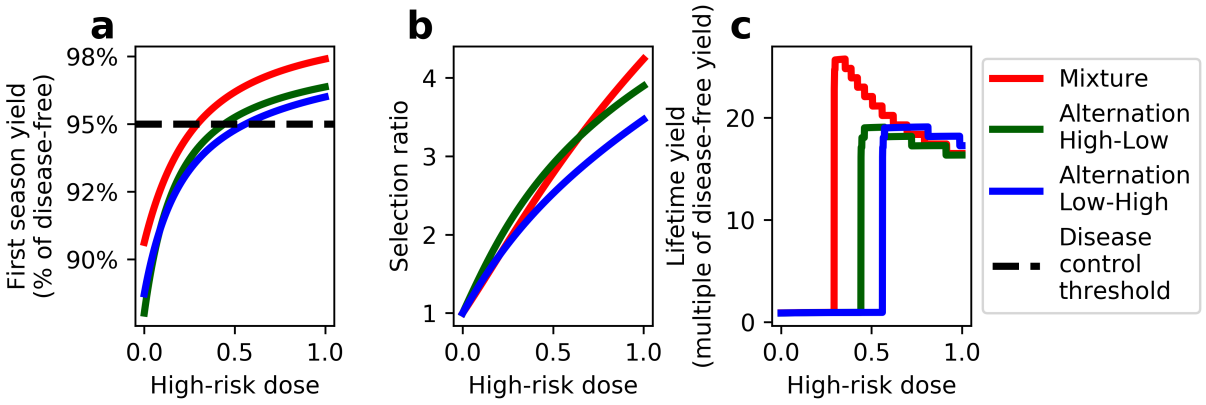

Figure 3.4: The performance of each tactic for a range of doses of the high-risk when the dose of low-risk to set to one. Performance is shown in terms of the **a)** yield in the first season, **b)** selection ratio and **c)** lifetime yield. The dashed line shows the critical level of disease control.

high-risk dose for a fixed dose of low-risk. We shall use the case of a full low-risk dose ($C_L = 1$), so that the response is equivalent to moving from the bottom right of the previously shown plots to the top right (figure 3.4). For any given dose of the high-risk (C_H), mixture leads to a higher initial yield due to dose-splitting (figure 3.4a). The implication is that a lower dose of the high-risk can maintain adequate control under mixture ($C_H \geq 0.29$; dashed line in figure 3.4c) compared to alternation ($C_H \geq 0.44$ or 0.56). The pattern for the response of selection to high-risk dose is more complex, with both mixture and alternation potentially leading to smaller selection ratios at different doses (figure 3.4b).

To understand optimum performance in more detail, we compare the selection ratios at the low end of permissible doses for each tactic, since these maximise lifetime yields (figure 3.4c). For the model and parameterisation used here, the lower permissible dose under mixture outweighs the effect of spraying the high-risk twice as often, and exerts less selection than the lowest permissible doses under either alternation. At 95% yield in the first season, the selection ratio is 2.28 for mixture compared to 3.08 and 3.15 for Alternation High-Low and Low-High respectively. The lower selection ratio leads to a longer effective lifetime, and spraying the fungicides as a mixture therefore optimises lifetime yield.

The optimal dose of the high-risk fungicide is slightly higher than the minimum dose ensuring acceptable control in the first season. This is because the effective lifetime is discrete, leading to ranges of dose which all break down within the same season. Within any range of doses with the same effective lifetime, the optimum lifetime yield is obtained by selecting one of the higher doses of high-risk, benefitting from slightly improved control in each season it remains effective. Too high a dose however can lead to more dramatic failure in the final season, and thus the optimal dose may not be the highest dose with the longest effective life. This is difficult to see in figure 3.4c, but the “horizontal” parts of the response are not in fact quite horizontal. The red arrow showing the optimal dose combination on figure 3.3b is therefore above the boundary between the grey and dark-green regions.

The prediction for yield shows jagged upper and lower smooth boundaries between areas where mixture and alternation perform better (figure 3.3b), whilst for resistance management the only boundary is smooth (figure 3.3a). The smooth boundaries are generated by the case where only one alternation tactic is capable of out-performing mixture, whilst the jagged boundary is caused by the interplay between the two alternation tactics. This is clearly visible by looking at the position of the curves for which a non-zero effective life is possible for each strategy in dose-space (figure 3.5g-i). In addition, the raw data clearly shows the expected trends with dose (figure 3.5). Increasing either dose increases the amount of disease control in the first season for all tactics. Increasing the dose of low-risk decreases the selection ratio greatly under mixture and slightly under either of the alternation tactics. That the low-risk dose has any effect on selection under the alternation tactics is because the decay rates of the fungicides are such that there is temporal overlap of chemicals sprayed at T1 and T2. For all tactics if not enough fungicide is applied, the lifetime yield is very low as acceptable yield is not produced in the first season. As doses are increased to moderate levels the first season yield is sufficient, and the lifetime yield gets significantly larger. As the high-risk dose is increased further, the additional disease control is offset by increased selection and the lifetime yield decreases. As the low-risk dose is increased further disease control increases and so the lifetime yield increases.

3.5 Difference between the alternation tactics

According to an explanation of tactic performance based purely on the effect of dose-splitting and suppression by the mixing partner, the two alternation tactics should perform identically. However, other effects lead to these tactics achieving different levels of resistance management and yield (figure 3.6). In particular we see that for almost all doses Alternation Low-High imposes significantly less selection, and for yield purposes Alternation High-Low performs better at lower doses of the high-risk and Alternation Low-High at higher. As these tactics apply exactly the same doses of each fungicide, the only possible mechanisms for this are related to timing or ordering of sprays. The ordering of sprays can have an effect as the decay rates of the fungicides are such that doses applied at different sprays overlap. As the low-risk and high-risk decay at different rates, the degree to which they overlap depends on the order of their application. Notably there is greater overlap, and thus greater suppression of selection by the low-risk under Alternation Low-High compared to Alternation High-Low (figure 3.7). In addition, as mentioned in chapter 2, the per capita growth rate of infectious tissue is larger at GS32 than GS39 and so, all else being equal, applying the high-risk at the T1 spray imposes more selection than at T2.

In a similar way to the comparison of alternation and mixture, the difference in lifetime yield between the two alternation tactics can be understood in terms of the trade-off between control and selection. As mentioned previously applying the high-risk at the T1 spray gives

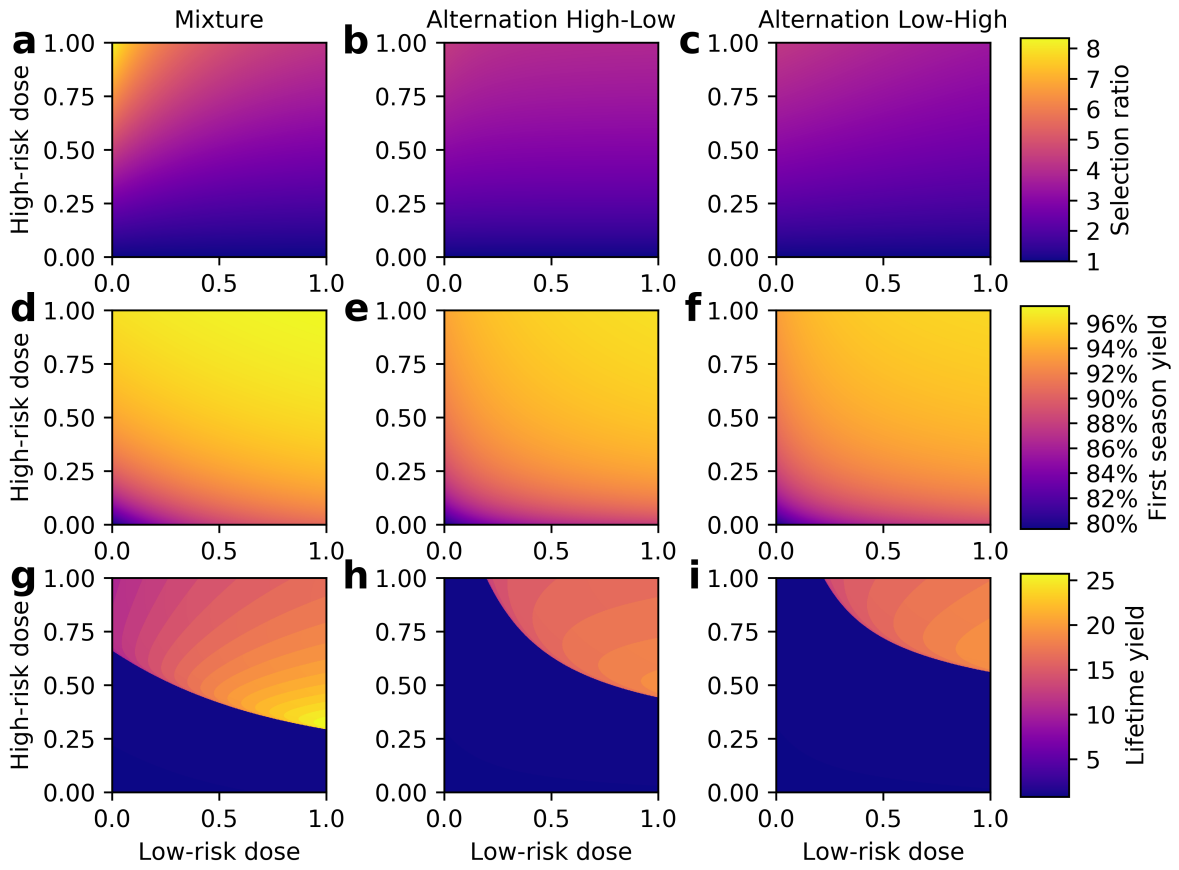

Figure 3.5: The raw data underlying the predictions of relative tactic performance in figure 3.3. Each row shows data for a different metric: **a-c)** selection ratio, **d-f)** the yield produced in the first season and **g-h)** lifetime yield. Each column shows the data for a different tactic: **a, d, g)** Mixture, **b, e, h)** Alternation High-Low and **c, f, i)** Alternation Low-High.

more control than at the T2. Therefore Alternation High-Low is able to produce an acceptable initial level of control at lower doses of either fungicide than Alternation Low-High, and so the former vastly out-performs the latter at these doses. However the increased control from the high-risk dose also means it imposes a greater selection pressure. Thus at higher doses, where increased control is less important than decreased selection, Alternation Low-High is preferred. The effect of timing will be revisited in later chapters.

3.6 Accounting for variation in disease control

There is a strong relationship between the level of disease control a tactic gives and the selection pressure it imposes. For a fixed total dose the different tactics impose different levels of control and thus selection pressures. Dose-splitting in mixture means the fungicides exert a greater effect, and effects related to spray timing mean that the alternation tactics give different levels of control for the same dose. This may make the previous comparisons with conserved dose an unfair test, especially given that growers are likely to be more interested

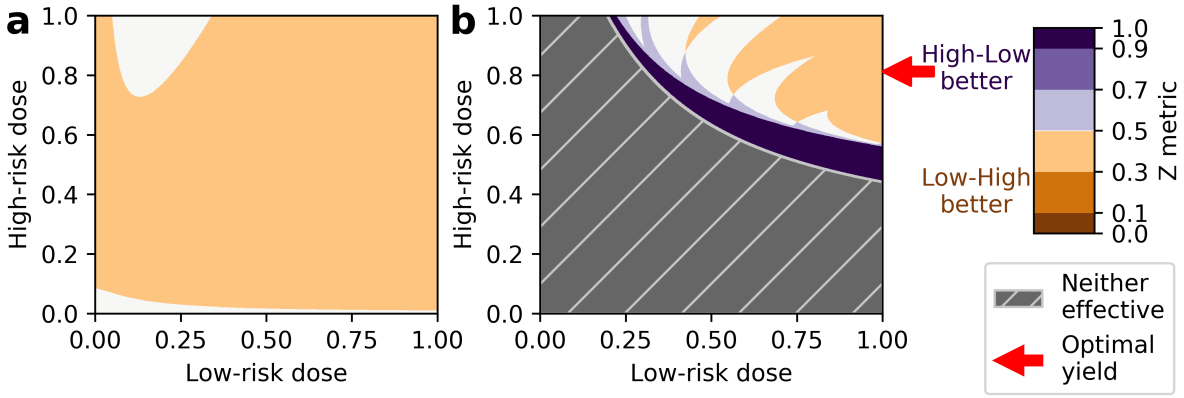

Figure 3.6: Relative performance of the two alternation tactics at a range of doses when considering **a)** selection ratio and **b)** lifetime yield. A value of the Z metric greater than 0.5 indicates that Alternation High-Low is better and lower Alternation Low-High. The Z metric is defined as $SR_{LowHigh}/\left(SR_{HighLow} + SR_{LowHigh}\right)$ in **a** and as $LY_{HighLow}/\left(LY_{HighLow} + LY_{LowHigh}\right)$ in **b**.

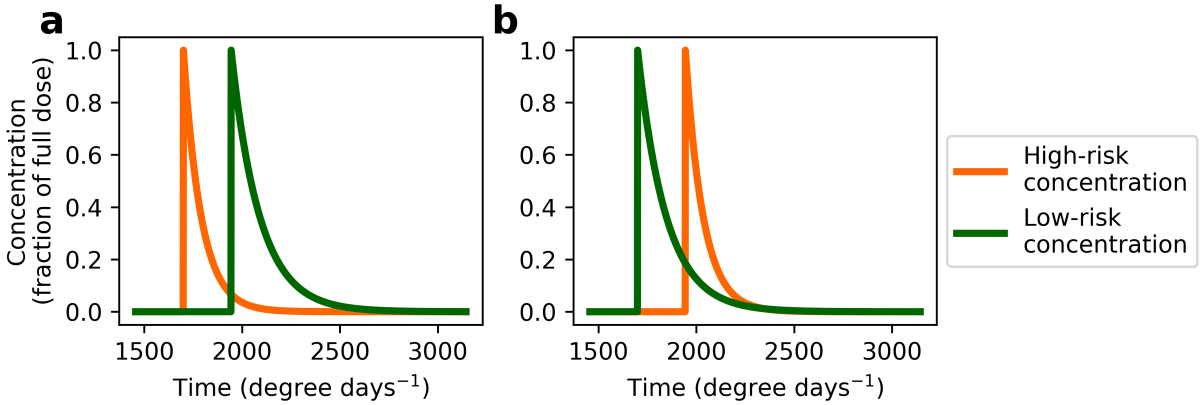

Figure 3.7: The concentration of the high-risk and low-risk over time when a full dose of each is applied under **a)** Alternation High-Low **b)** Alternation Low-High.

in the best tactic for a given level of disease control rather than at a fixed dose. The lack of influence on agronomic practice of earlier models has in part been attributed to the lack of consideration of the levels of disease control provided by different tactics (van den Bosch *et al.*, 2015). Due to the difficulty of predicting the exact disease control a tactic will provide, we re-examine the data generated for the varying dose comparison removing the conserved dose constraint (figure 3.8).

The first observation from these data is that there is large variation in the lifetime yield generated and selection pressure imposed by the tactics for a given level of control. One obvious mechanism for this is that the same level of control can be achieved under the same tactic by varying the ratio of low-risk to high-risk fungicide used. When more low-risk fungicide is used, the selection pressure can only be the same or lesser and the lifetime yield the same or greater. For any given level of control, mixture is capable of generating a lower selection ratio and a higher long-term yield. However, mixture is also capable of producing greater selection pressures and lesser lifetime yields than both alternation tactics

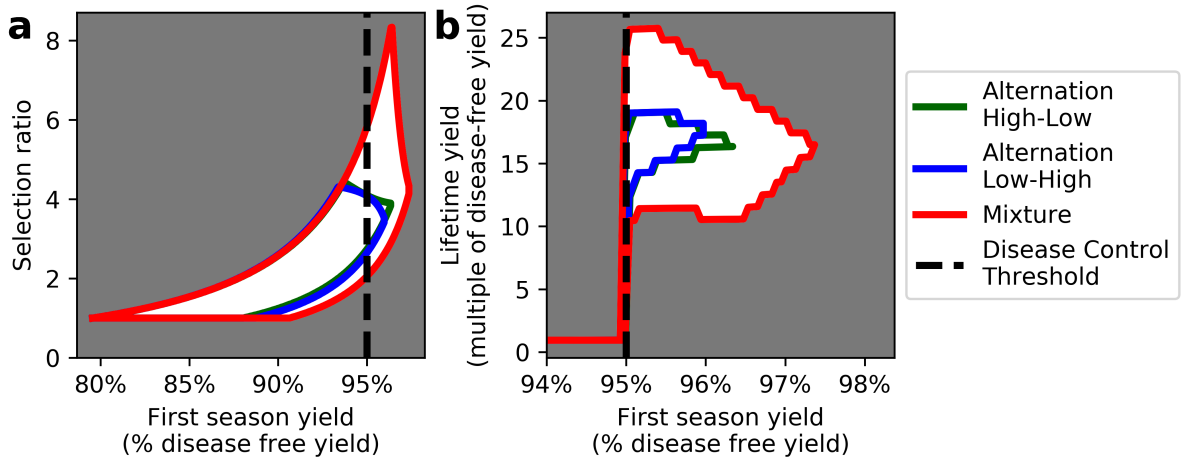

Figure 3.8: Comparison of mixture and alternation tactics without the constraint of applying the same total dose. The performance of the tactics is shown in terms of **a)** selection ratio and **b)** lifetime yield. A range of values can be produced by each tactic for any given level of control; the polygons show the range that these values fall within for each tactic.

for any given level of control. As seen in the comparisons at fixed total dose, the overall best lifetime yield is achieved by mixture when spraying only slightly more fungicide than required to get acceptable disease control in the first season.

3.7 Discussion

3.7.1 Main conclusions

In this chapter, we have used a model of septoria leaf blotch on winter wheat to investigate which of mixture or alternation of a single high-risk and low-risk fungicide are superior for resistance management and long-term yield. Mixture was shown to perform relatively better than alternation when the applied dose of the high-risk fungicide is low. This is true when the tactics are compared in terms of resistance management or long-term yield. For resistance management purposes, it is also generally true that mixture performs better compared to alternation when the dose of the low-risk is higher. For long-term yield the relationship between low-risk dose and relative tactic performance is more variable and complex.

3.7.2 Mechanisms

The overall superior performance of mixture is in large part due to the effect of dose-splitting. Dose-splitting of the low-risk fungicide means that mixture can produce better disease control even without the use of the high-risk fungicide. This means that mixture can use lower doses of the high-risk than alternation, and thus impose lesser selective pressures, whilst still maintaining disease control. As an additional benefit, during the time period that the

high-risk dose is applied in mixture the growth rate of the resistant strain is suppressed by the low-risk, reducing selection.

3.7.3 Previous literature

That either mixture or alternation can perform better for resistance management depending on the exact situation fits well with the existing modelling literature, where either tactic was found to perform better depending on spray coverage (Josepovits & Dobrovolszky, 1985; Kable & Jeffery, 1980), fungicide effectiveness (Josepovits, 1989), fungicide decay rates (Josepovits & Dobrovolszky, 1985; Levy *et al.*, 1983), base infection rates (Josepovits & Dobrovolszky, 1985), level of synergy between fungicides (Levy *et al.*, 1983), level of resistance (Skylakakis, 1981) or latent period length (Skylakakis, 1981). Most of these papers favoured mixture in general, with alternation only performing better under certain conditions. For the case of the same total dose being applied between mixture and alternation, our results point toward a more balanced situation. When the level of control is conserved instead of the dose applied, our results agree with the literature that mixture is generally better than alternation.

The overall optimal performance of mixture is in agreement with the most recent modelling paper on mixture and alternation, although this is hardly surprising as the model used in that paper is almost identical to our own (Hobbelen *et al.*, 2013). However, our findings do support that the good performance of mixture in that paper is dependent neither on the mixing partner being a high-risk fungicide nor the exact fungicides modelled. This result can not be readily compared with the earlier literature, as metrics taking into account both yield production and resistance development were not used (van den Bosch & Gilligan, 2008).

The tactic of applying as much low-risk fungicide as possible and a little more than the minimal amount of high-risk fungicide required for initial control has been identified multiple times in the literature before (Hobbelen *et al.*, 2011a; van den Bosch *et al.*, 2014b). This is unsurprising as the consensus is that a reduction in dose reduces selection for resistance (van den Bosch *et al.*, 2011), and low-risk fungicides are often modelled as being effectively "free" in that they grant control with no selection for resistance.

3.7.4 Implications for practice

Our analyses showed that when the fungicides were applied at full dose, alternation is the superior tactic for both resistance management and long-term yield. However, this result is not general and depends on the exact epidemiological context, for example the fungicides used. In addition, it may be that legal fungicide dose restrictions are per-spray rather than over the season, and so mixture may be permitted to apply higher doses than used in our full dose analysis. Ultimately we present this result as evidence that alternation can out-perform mixture when doses are high, rather than as a suggestion that alternation should be used in

practice.

Fungicides are sprayed at less than their full label dose in many cases to cut costs and reduce the risk of resistance development (Jørgensen *et al.*, 2017). Therefore the variable dose comparisons we carry out may be more applicable to agronomic practice than the full dose. The comparisons at conserved total dose between alternation and mixture are not particularly realistic, as growers are likely not very interested in the particular volume of fungicide they apply if increased yields can account for any extra fungicide costs. These comparisons are however a useful aide to understanding.

One potential issue with the optimal tactic identified is that it relies on applying only slightly more fungicide than needed for acceptable control. The implication is that if estimates of fungicide performance are inaccurate, or disease is more severe than expected, then this tactic could lead to failure of control. To avoid such failure, growers may be risk-averse and apply more fungicide than strictly needed for control (te Beest *et al.*, 2013). Depending on the level of uncertainty in disease pressure or fungicide performance, risk aversion may lead to doses being applied that would be better used under alternation. On the other hand, mixture has a built-in insurance mechanism in that if the high-risk fails, the low-risk still provides some control at every spray (Shaw, 2006). However, it was seen that choosing mixture doses poorly was capable of giving much worse long-term yield than the worst possible alternation. This is due to the high cost of dose-splitting when too much high-risk fungicide is applied, and presents another reason that a risk-averse grower may prefer alternation.

The difference in performance between tactics was in many cases relatively small, for example at full dose there was only a single season's difference in the effective lives between tactics. However these differences may in practice may be expanded by factors that would be expected to increase the effective lives of all tactics by the same factor, such as smaller initial resistance frequencies, less aggressive disease progression or crop rotation.

Throughout we have assumed that the initial frequency of resistance is 10^{-10}. This is lower than typical rates for point mutations in fungal genomes, and thus might be seen as too low a value. However, the level of resistance in a fungicide-naïve fungal population is generally too low to measure (Walker *et al.*, 2017), making this parameter practically unknowable. A recent paper estimated resistance mutation rates at low as 10^{-18}, suggesting this may be lower than expected due to requiring secondary enabling mutations for high levels of resistance, or that multiple copies of mitochondrial mutations may be required (Mikaberidze *et al.*, 2017).

It is worth noting that we did not carry out an economic analysis as the monetary cost of fungicide was ignored, although this is partially covered in the cases where the total dose of each fungicide is conserved. In addition we did not consider other economic factors such as discounting. This was considered to be outside the scope of this work, but could be added in future analyses.

Chapter 3 Summary

- Mixture and alternation are two tactics for managing fungicide resistance when a high-risk and a low-risk fungicide are available.
- These tactics were compared with a model of septoria leaf blotch of UK winter wheat.
- When applying the same total dose, either tactic can perform better for resistance management or long-term yield.
- The overall optimal tactic for lifetime yield was to apply a mixture with as much low-risk fungicide as possible and slightly more high-risk than required for initial disease control.

4

Testing the robustness of the mixture and alternation comparison

> *Bring me a magic potion; it will heal my aching wounds. A taste so bitter that makes my bleeding soul feel so good.*
>
> Ensiferum, *One More Magic Potion*, 2007

4.1 Introduction

In the previous chapter we compared the performance of fungicide mixture and alternation using a model of septoria leaf blotch on UK winter wheat. In this chapter we examine the robustness of the results of that comparison to the following factors.

- Parameter values.
- The pathosystem modelled.
- The presence of resistance to the mixing partner.
- Partial resistance to the high-risk fungicide.

4.2 Robustness to parameter values

4.2.1 Nature of analysis

The first and simplest check of robustness we carry out is to examine the sensitivity of the results to the parameter values used in the model. For this analysis we examine the qualitative sensitivity of the model to a number of parameters in isolation. We could have carried out a quantitative analysis, such as with sensitivity indices (Saltelli *et al.*, 2004), but we are more interested in the broad conclusions rather than the values of the numeric outputs.

For the sensitivity analysis we fixed the low-risk dose at full dose (half for mixture) as simple reasoning, validated by previous results, shows this to always give the best tactic performance by any metric we use. We also do not examine the full range of parameters in the model but instead choose a subset of particular interest.

4.2.2 General patterns

Across all parameter values investigated, the same characteristic patterns of tactic performance were seen (figures 4.1 and 4.2). At lower doses of the high-risk fungicide mixture performs better and at higher doses alternation, for both resistance management and long-term yield. In general, if the high-risk fungicide was made more effective (increasing the maximum effect or curvature parameter, or decreasing its decay rate) then alternation performs better over a wider range of doses for both resistance management and yield. This is because increasing the effect of the high-risk further increases the selective cost of dose-splitting in the mixture. We might expect that increasing the effect of the low-risk would favour mixture, as only mixture is expected to receive the suppressive effect on selection from the mixing partner. However this is not always the case. This is because the decay rates of the fungicides are such that there is overlap of fungicides sprayed at different times, and so alternation receives some suppressive effect too. In addition, the complex effects of timing make the relationship between low-risk effectiveness and tactic performance less simple.

4.2.3 Effect of epidemiological parameters

It is notable that the infection rate, latent period and initial frequency of resistance have very little effect on the relative tactic performance for resistance management. The values of these parameters are uncertain and likely vary between seasons and locations, so it is very encouraging that they have little effect. The epidemiological parameters have a much larger effect on the lifetime yield comparison, as they affect the degree of control needed for acceptable yield. Increasing the amount of control required shifts the characteristic pattern up the high-risk dose axis, and thus favours mixture as it performs better toward the bottom of this pattern.

4.2.4 Random parameter space search

So far we have checked the sensitivity of our predictions to each parameter in turn, but have not investigated interactions between pairs of parameters or other higher-order effects. Attempting to do with dense scans over parameter space as before quickly becomes computationally unfeasible and difficult to present in a meaningful way. Therefore we investigate performance across parameter space via a randomisation algorithm (algorithm 4.1) and check whether each parameterisation leads to mixture or alternation giving the overall optimal lifetime yield. For each of 1000 random parameterisations we found no case where mixture did not produce the overall best lifetime yield. The algorithm used has a built-in check for realism of a parameter set by checking performance at two key pairs of fungicide doses. It first checks that acceptable control is not possible when applying the low-risk fungicide

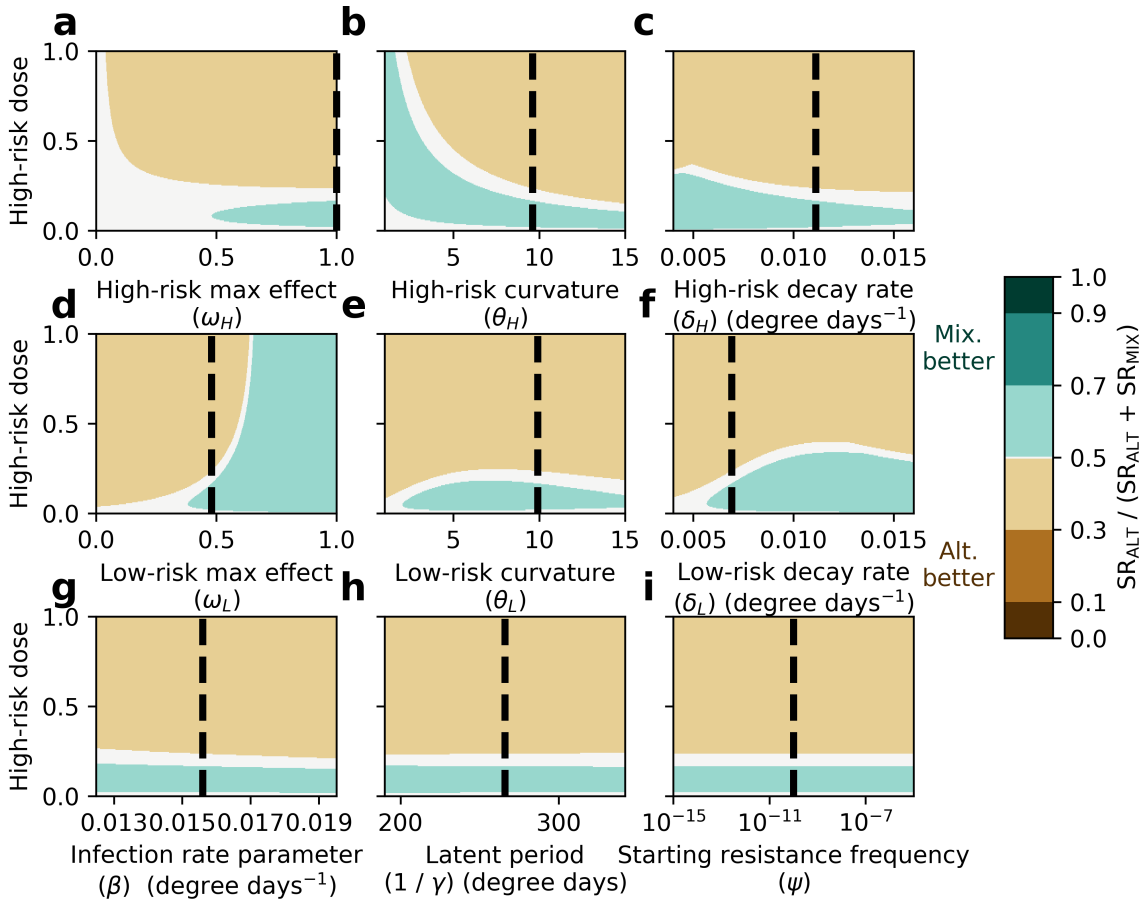

Figure 4.1: The relative performance of mixture and alternation in terms of the selection ratio at a full dose of low-risk and a variable dose of high-risk. Each panel shows the response of the metric to a different parameter's value. The dashed line shows the value used for the parameter in the rest of this chapter. The parameters are: **a)** maximum effect of the high-risk, **b)** curvature parameter for the high-risk, **c)** decay rate of the high-risk, **d)** maximum effect of the low-risk, **e)** curvature parameter for the low-risk, **f)** decay rate of the low-risk, **g)** infection rate parameter, **h)** latent period, and **f)** the initial frequency of resistance.

alone ($C_L = 1$, $C_H = 0$) and secondly that acceptable control is possible when applying both fungicides at full dose ($C_L = 1$, $C_H = 1$). Roughly half of all parameter sets tested passed this test and were used for the analysis.

4.3 Robustness to pathosystem

4.3.1 Nature of analysis

A more exacting test of the generality of our results is to carry out the same analysis for a model of a different pathosystem. For this we use the model of powdery mildew on grapevine described in section 2.5. The modelled high-risk fungicide is trifloxystrobin and the low-risk sulphur.

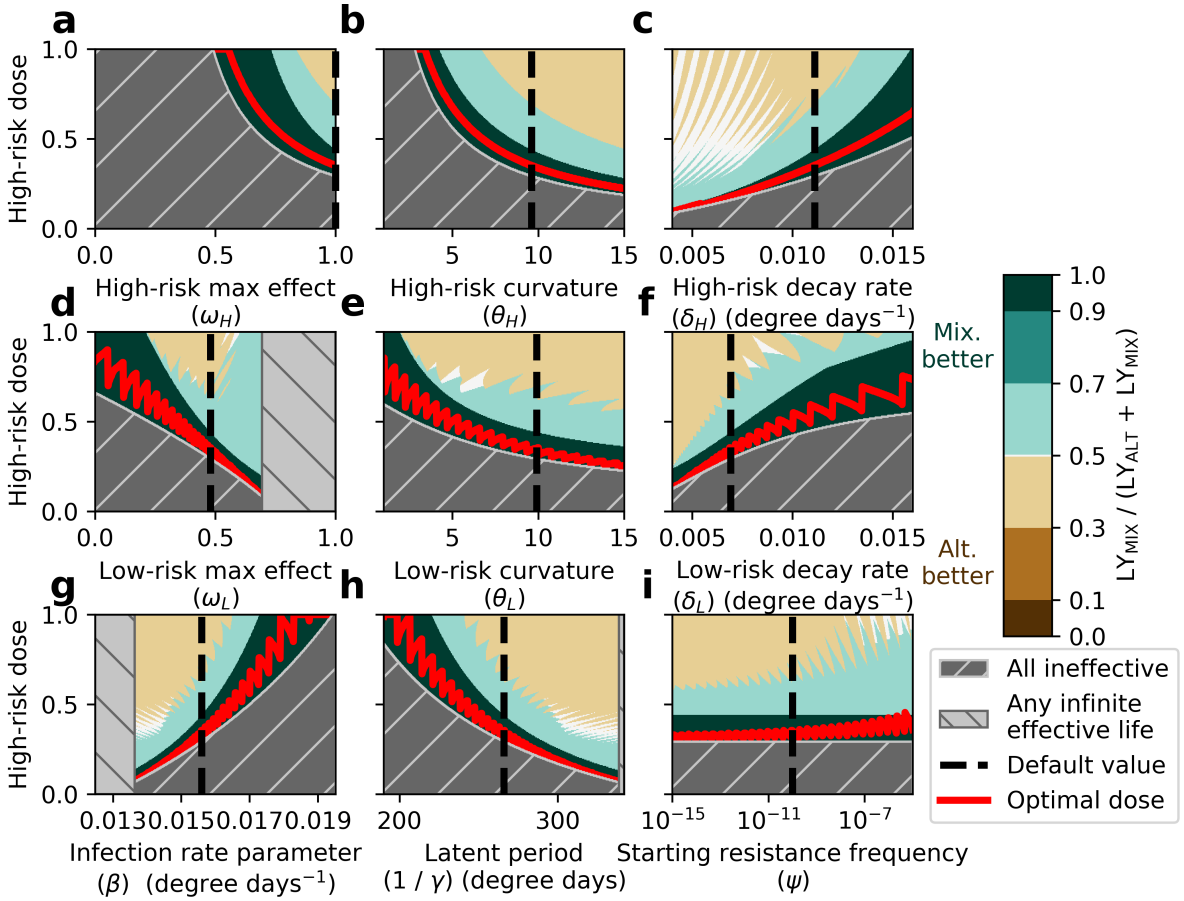

Figure 4.2: Relative tactic performance in terms of lifetime yield at a full dose of low-risk and a variable dose of high-risk. Each panel shows the response of the metric to a different parameter's value. Areas where either all tactics fail to achieve a single season of acceptable yield or where any tactic has an infinite effective life are shaded out due to representing unrealistic areas of parameter space. The dashed line shows the value used for the parameter in the rest of this chapter. The red line shows the dose leading to the highest lifetime yield for any value of the parameter. The parameters investigated are: **a)** maximum effect of the high-risk, **b)** curvature parameter for the high-risk, **c)** decay rate of the high-risk, **d)** maximum effect of the low-risk, **e)** curvature parameter for the low-risk, **f)** decay rate of the low-risk, **g)** infection rate parameter, **h)** latent period, and **f)** the initial frequency of resistance.

4.3.2 Full dose

As before we first examine the performance of the two tactics at full dose (figure 4.3). As with the septoria model, alternation imposes a lesser selection pressure and permits a larger lifetime yield, measured by effective life rather than lifetime yield for this pathosystem (see section 2.7). Yet again mixture provides better disease control when resistance is near fixation than either alternation tactic. The difference in initial disease control between Alternation High-Low and mixture is much smaller than in the septoria case, identifying the importance of the first spray for disease control in this system. The change in seasonal disease severity (related to yield in the septoria model) and resistance frequency over time

Algorithm 4.1 Comparing mixture and alternation for random parameter values

$N \Leftarrow 0$
$X \Leftarrow 0$
while $N < 1000$ **do**
  Choose parameter values randomly from uniform distributions with ranges displayed in figure 4.1.
  Run simulation with chosen parameters for a single season with $C_H = 0$ and $C_L = 1$, $T_1 \Leftarrow$ yield over that season.
  Run simulation with chosen parameters for a single season with $C_H = 1$ and $C_L = 1$, $T_2 \Leftarrow$ yield over that season.
  if $T_1 < 0.95$ AND $T_2 \geq 0.95$ **then**
    $N \Leftarrow N + 1$
    Run simulations for each tactic with $C_L = 1$ and C_H in [0, 1]
    if Best lifetime yield given by mixture **then**
      $X \Leftarrow X + 1$
    end if
  end if
end while
X/N gives the proportion of times mixture was optimal

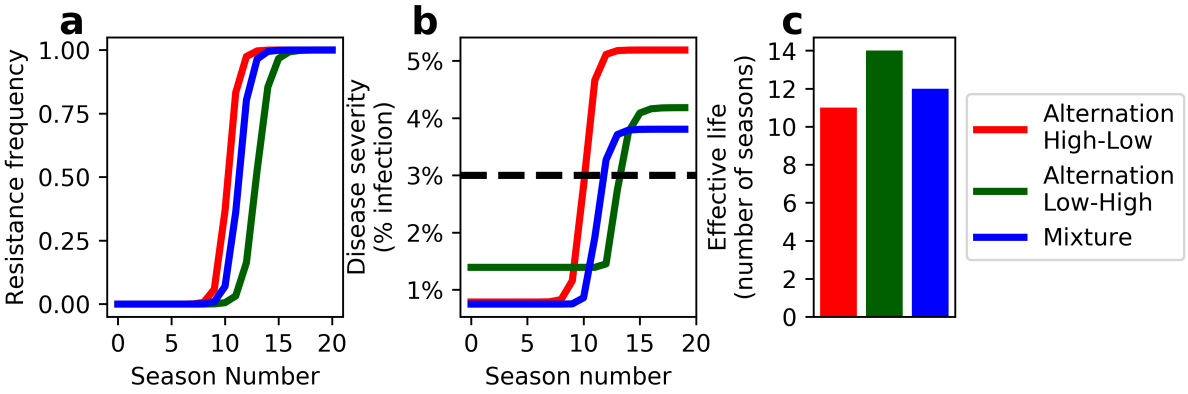

Figure 4.3: The performance of each tactic at full dose (half for mixture) in the model of powdery mildew. **a)** The frequency of resistance at the start of each season. **b)** The yield produced each season. **c)** The effective life under each tactic.

look very similar to the dynamics in the septoria model.

4.3.3 Variable dose

We now investigate the results of the powdery mildew model when fungicides are sprayed at less than full dose (figure 4.4). Compared to the septoria model, the prediction of which tactic is better is not necessarily the same between the two models for the same dose, but the same general pattern holds (cf. figure 3.3). Mixture performs better at lower doses of the high-risk and alternation better at higher for resistance management. For lifetime yield alternation performs better at higher doses of the high-risk and the pattern with low-risk

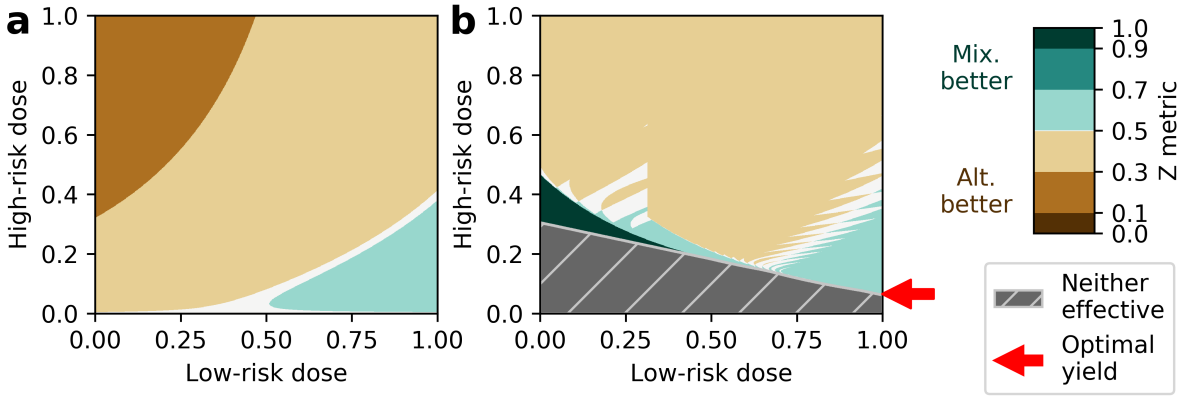

Figure 4.4: Relative performance of mixture and alternation for a variety of dose combinations in the powdery mildew model. A higher value of the Z metric implies that mixture performs better and a lower alternation. A value of 0.5 means that the tactics are performing equally well. Predictions are shown based on **a)** selection ratio and **b)** effective life.

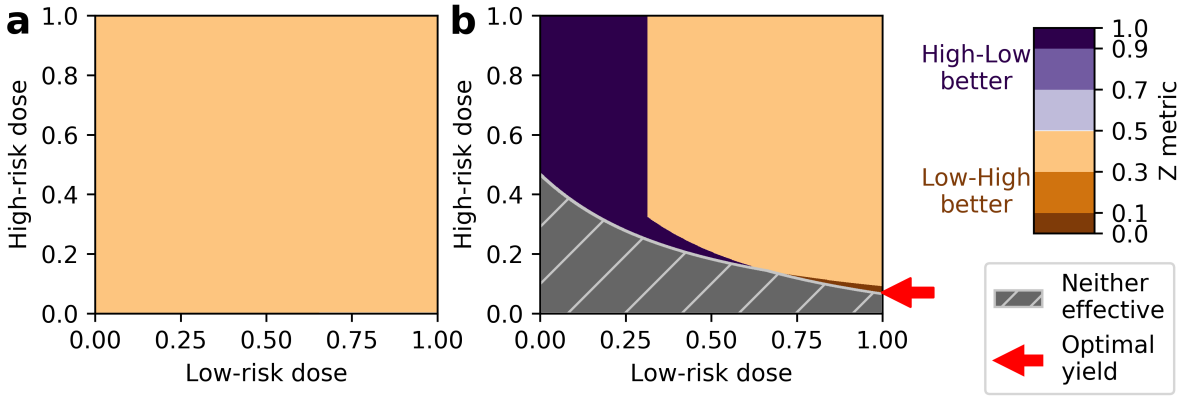

Figure 4.5: Relative performance of the two alternation tactics for a variety of dose combinations in the powdery mildew model. A higher value of the Z metric implies that Alternation High-Low performs better and a lower Alternation Low-High. A value of 0.5 means that the tactics are performing equally well. Predictions are shown based on **a)** selection ratio and **b)** effective life.

dose is more complex. The shape of the boundary between areas where mixture and alternation perform better for lifetime yield is produced by the pattern of performance for the two alternation tactics. Below a low-risk dose of around 0.3 the Alternation Low-High tactic cannot produce sufficient yield to permit a non-zero effective life, again highlighting that control at the first spray is very important (figure 4.5). This means that below that dose of low-risk mixture is competing with Alternation Low-High tactic and above with Alternation High-Low, explaining the biphasic pattern in the mixture alternation comparison.

The boundary between the areas where mixture and alternation perform better for resistance management curves in the opposite direction to the septoria model, concave upward rather than concave downward. The mechanism behind this will be revisited later in section 5.2.

4.3.4 Accounting for variation in control

Finally we relax the constraint of only comparing tactics at the same dose, and instead examine the relationship between tactic performance and the amount of disease control granted (figure 4.6). The pattern is strikingly similar to the septoria model (figure 3.8), where mixture can produce lower selection ratios and longer effective lives for any given level of disease control. There is a slight difference to the septoria results in that, for some levels of disease control, the range of values produced by the alternation tactics do not fall within the range of those of mixture. This is because of the greater effect of spray timing in this model, which alternation is able to make better use of. However, in general the alternation tactics do produce intermediate selection ratios and effective lives for a given level of control compared to the extremes possible under mixture.

There is a very noticeable “kink” in the relationship between the selection ratio imposed by the Alternation Low-High tactic and the level of control it provides (figure 4.6). This is because of the timing of the two sprays relative to the critical period of time over which infection severity is measured. The second spray happens late in the critical period, 12 days into the 30 day period. The earlier spray therefore has a much larger impact on the level of disease control over this period. However the impact on selection for resistance from the second spray can still be large as the season continues after the critical period. The position of the kink at around 5% infection severity represents the maximal level of control that applying the high-risk alone permits under Alternation Low-High. Then adding any low-risk at all leads to a very different relationship between control and selection, as the earlier spray imposes a lot of control but increasing the dose of low-risk has little effect on selection. A similar kink is not seen in the data for the Alternation High-Low tactic as applying the high-risk at the first spray does not lead to the same decoupling of control and selection.

4.4 Robustness to the presence of resistance to the mixing partner

4.4.1 Nature of analysis

We have assumed thus far that there is a negligible chance of resistance arising to the mixing partner that is used. For fungicides such as chlorothalonil and sulphur this is likely a good assumption, but it is important to test if our main conclusions are conditioned on this assumption. This is an important test both as it may be that resistance will eventually arise to fungicides we consider low-risk, and also it is useful to know if the same tactics perform better when using two high-risks as when using a low-risk. We consider here both the FiveLeaf septoria and powdery mildew models.

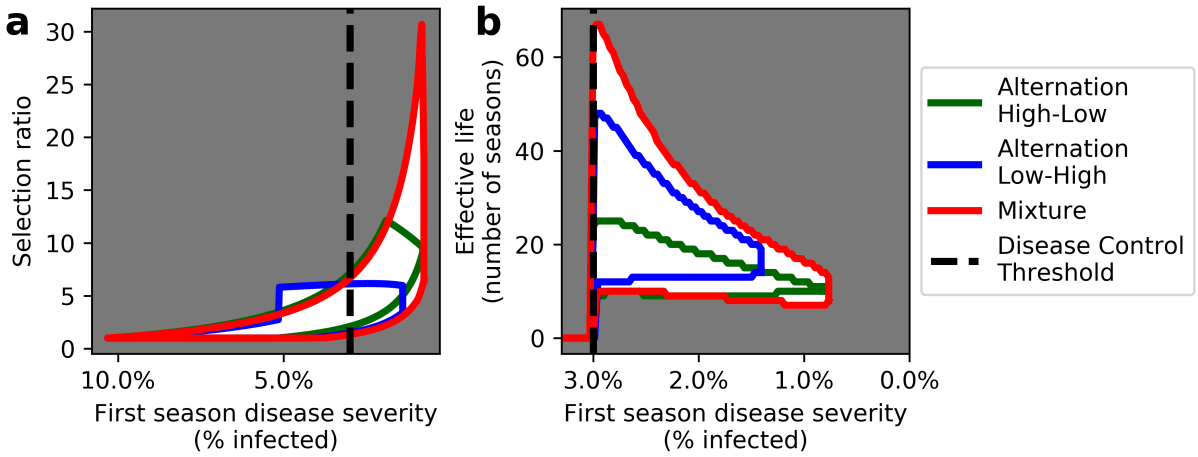

Figure 4.6: Comparison of mixture and alternation tactics in the powdery mildew model taking into account the level of disease control they provide. The performance of the tactics is shown in terms of **a)** selection ratio and **b)** effective lifetime. A range of values can be produced by each tactic for any given level of control; the polygons show the range that these values fall within for each tactic.

We use the same dose-response curves for the fungicides as before, although resistance to the mixing partner is also now present. To avoid confusion will now refer to what was the high-risk fungicide as the primary fungicide and what was the low-risk as the partner fungicide. Modelling resistance to both fungicides requires 4 different pathogen strains,

1. SS which is sensitive to both fungicides (the same as the sensitive strain used elsewhere in this chapter)
2. RS which is resistant to the primary fungicide and sensitive to the partner (the same as the resistant strain used elsewhere)
3. SR which is sensitive to the primary fungicide but resistant to the partner
4. RR which is resistant to both fungicides

The septoria and powdery mildew models were trivially adapted to include these 4 strains. For the septoria model, the comparison of mixture and alternation with resistance developing to both fungicides is thus very similar to the work in Hobbelen *et al.* (2013).

It is still assumed that there is no mutation or sexual reproduction, and also that the initial frequency of the double resistant stain is the product of the initial frequencies of the two singly-resistant strains. The same metrics can be used to evaluate the tactics as before, although there is now selection for multiple strains occurring. Ultimately we are interested in how selection impacts fungicide performance, rather than pathogen population structure, and so we choose to quantify resistance by selection for resistance to the two fungicides rather than for a given strain. The resistance frequency for a given fungicide is calculated by determining the population average value of the resistance trait for that fungicide (see

section 2.3 for brief explanation of the resistance trait). For example the resistance frequency for the primary fungicide at time t is given by

$$\phi_{\text{PRI}} = \frac{I_{RR}(t) + I_{RS}(t)}{I_{RR}(t) + I_{RS}(t) + I_{SR}(t) + I_{SS}(t)}\,. \tag{4.1}$$

4.4.2 Variable dose

We compare the tactics under the constraint that they apply the same total dose of each fungicide (figures 4.7 for septoria and 4.8 for powdery mildew). It is clear that selection for resistance against the primary fungicide is largely unaffected by selection against the partner (compare panel a of figures 3.3 and 4.4 against panel b of figures 4.7 and 4.8 respectively). The pattern of tactic performance in dose-space with regards to resistance management is inverted for the partner compared to the primary. The primary fungicide can be thought of as a mixing partner for the partner, and so it makes intuitive sense that the axes should be swapped compared to the standard pattern. Despite selection acting against the mixing partner, the overall pattern of tactic performance with respect to long-term yield is very similar. That the yield pattern is not also inverted is because in both models the partner fungicide is less effective than the primary for a given dose, and so the dose of the primary has a larger effect on yield than that of the partner.

In general mixture will favour the less effective of the two fungicides, and alternation the more effective. This is because the effect of dose-splitting is larger for more effective fungicides, and so dose-splitting is beneficial for the less effective fungicide. Dose-splitting of the more effective fungicide suppresses the growth of the strain resistant to the less effective more than dose-splitting of the less effective increases its growth relative to the sensitive.

The overall pattern for yield performance is the same, but the overall optimal tactic changes slightly when resistance can develop to the partner fungicide. The optimal tactic is still to apply just a little more of the primary fungicide than necessary for initial control and to do so under mixture, but the optimal dose of the partner is now less than a full dose. The optimal tactic is decided by the relative effectiveness of each fungicide, as the less effective fungicide is “sacrificed” to protect the more effective. The optimal tactic in the powdery mildew model involves applying much more similar doses of each fungicide as the partner fungicide in that case has an efficacy more similar to the primary.

4.5 Partial resistance

4.5.1 Nature of analysis

A further implicit assumption throughout this work has been that resistance to the high-risk fungicide is full; the fungicide either has its full effect or none at all on a given strain. However

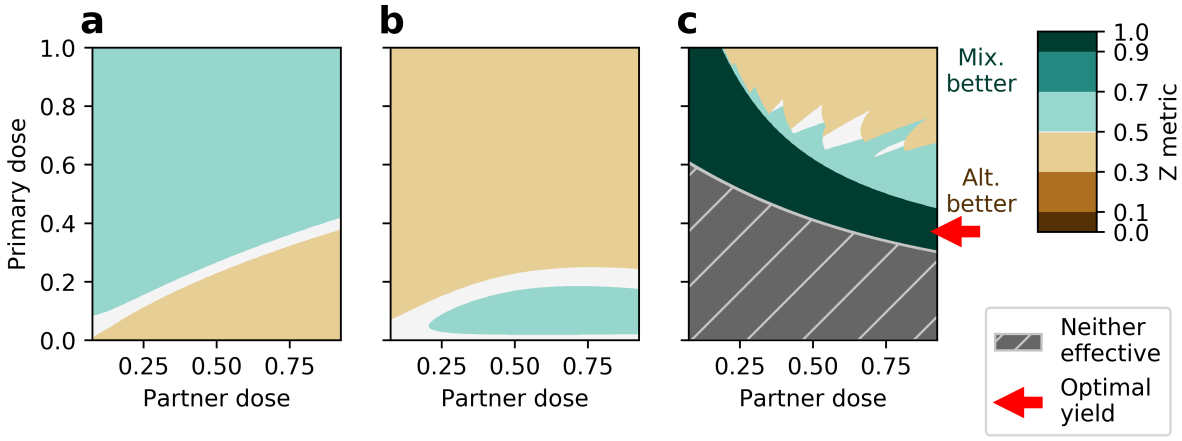

Figure 4.7: Relative performance of mixture and alternation for a variety of dose combinations in the septoria model when resistance is developing to both fungicides. A higher value of the Z metric implies that mixture performs better and a lower alternation. A value of 0.5 means that the tactics are performing equally well. Predictions are shown based on **a)** selection ratio for strains resistant to the partner fungicide, **b)** selection ratio for strains resistant to the primary fungicide, and **c)** lifetime yield.

is it possible that resistance will manifest as only a partial reduction in sensitivity, this is typical for azole resistance for example (Mikaberidze *et al.*, 2017). We consider two forms of partial resistance, and follow Mikaberidze *et al.* (2017) in referring to them as Type 1 and Type 2 partial resistance.

Type 1 Reduction in the maximum effect of the fungicide on a strain (ω).

Type 2 Reduction in the curvature parameter of the dose-response curve for that strain (θ).

Different degrees of resistance are modelled by different degrees of reduction in the appropriate parameter (section 2.3). Note that due to the nature of these types, strains with the same value of the resistance trait but different resistance types will not necessarily show the same response to fungicide.

4.5.2 Variable dose

We firstly examine the effect of partial resistance on the fixed dose comparisons for resistance management (figures 4.9 and 4.10). For both the powdery mildew and septoria models, Type 1 resistance (affecting the maximum effect of the fungicide) has no effect on the qualitative pattern of tactic performance for resistance management in dose-space. The degree of resistance just scales the difference between tactics, with a higher level of resistance leading to greater difference between tactics (a value of the Z metric further from 0.5).

Type 2 resistance has a minor effect on the balance of tactic performance for resistance management. As the degree of resistance decreases, the area where mixture out-performs alternation gets smaller. The degree of resistance can be thought as a dilution of the

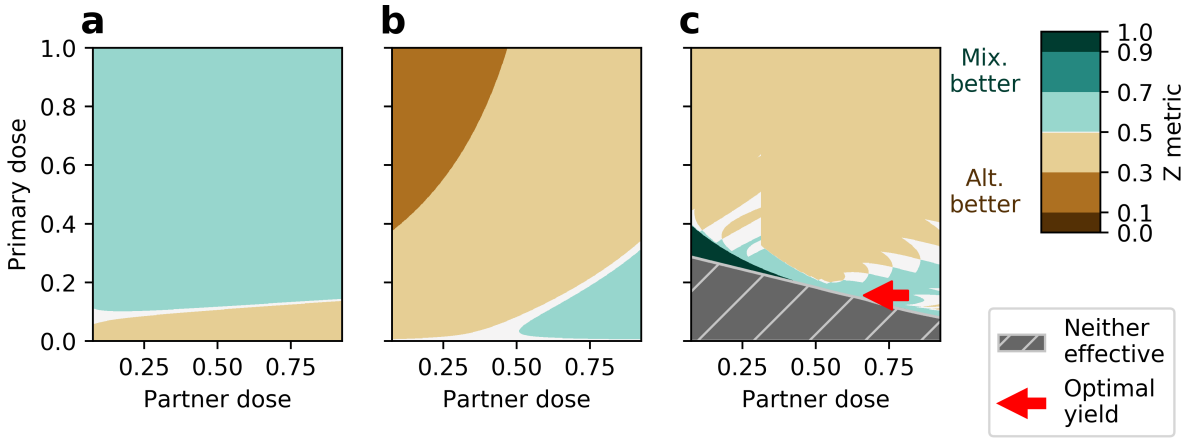

Figure 4.8: Relative performance of mixture and alternation for a variety of dose combinations in the powdery mildew model when resistance is developing to both fungicides. A higher value of the Z metric implies that mixture performs better and a lower alternation. A value of 0.5 means that the tactics are performing equally well. Predictions are shown based on **a)** selection ratio for strains resistant to the partner fungicide, **b)** selection ratio for strains resistant to the primary fungicide, and **c)** effective life.

high-risk fungicide concentration; the greater the resistance, the greater the dilution. As alternation tends to perform better at higher doses of the high-risk fungicide, it makes sense that decreasing the degree of resistance and thus decreasing the effective dilution of the high-risk fungicide also therefore favours alternation.

The effect of partial resistance is much more noticeable when looking at lifetime yield rather than resistance management (figures 4.11 and 4.12). The same general pattern as with full resistance is seen with regards to tactic performance at a particular dose, with mixture performing better at lower doses of the high-risk and alternation at higher. However, as the degree of resistance decreases it becomes possible at higher doses of the high-risk for tactics to achieve infinite effective lives. This is because even when resistance is at fixation the reduced effectiveness of the high-risk is still sufficient to provide acceptable disease control. Infinite effective lives are unlikely to be possible in reality however. The areas of dose-space where either tactic performs best are strikingly similar independent of the degree of resistance.

In all cases the optimal tactic is to apply as much low-risk as possible, as with full resistance. However the optimal dose of high-risk fungicide is affected by the magnitude and type of resistance. In most cases investigated the optimal tactic is to apply just a little more high-risk than is required for initial control in a mixture. However when the degree of resistance is low, and resistance is Type 2, it can become optimal to apply more high-risk fungicide and to do so under alternation. This is because when resistance is Type 2 rather than Type 1, the dose-response curves for the sensitive and resistant strains converge at higher doses. Thus an increased dose of high-risk does not necessarily increase the strength of selection (van den Bosch *et al.*, 2011). The dose-response curves for the resistant and

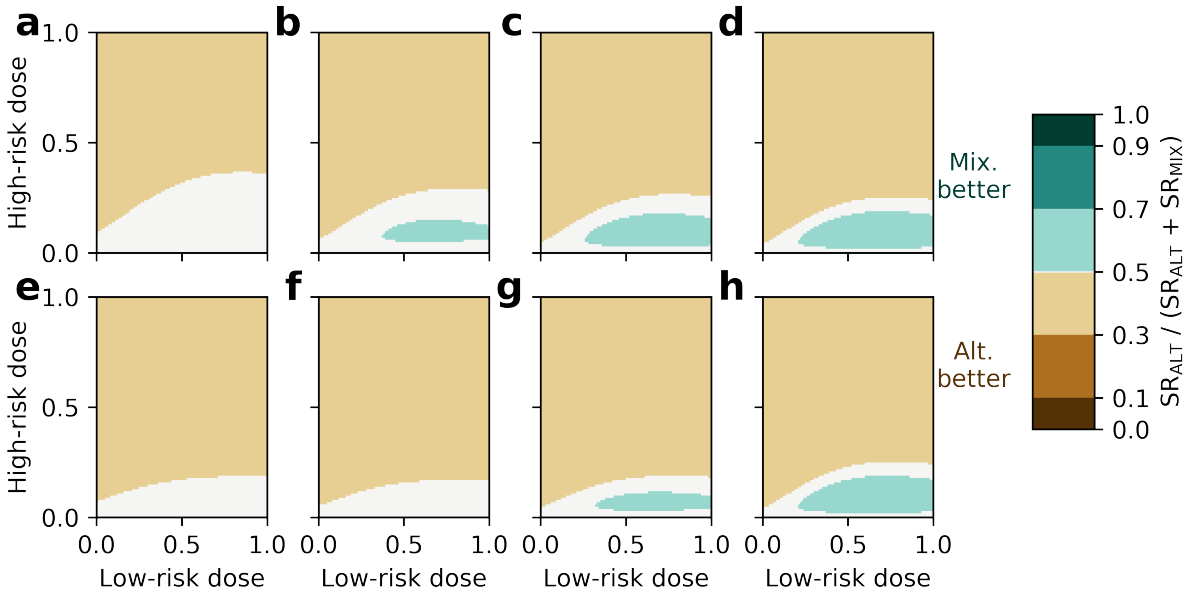

Figure 4.9: Relative performance of mixture and alternation for a variety of dose combinations in the septoria model when resistance to the high-risk fungicide is partial. Predictions shown are based on selection ratio. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1.

sensitive strains are clearly more similar the lower is the degree of resistance, and so we expect the effect of convergence to be more important.

Note that we explicitly remove the area where either tactic can give an infinite effective life from our consideration of optimality, and so it could be argued that identifying an increased optimal dose of high-risk is an artefact related to the boundary between tactics that give an infinite effective life and those that do not. However, there are cases where there is an area of infinite effective life at high dose and yet the optimal tactic does not sit on the boundary (*e. g.* figure 4.12a).

4.5.3 Accounting for variation in control

When we compare the tactics without requiring that they apply the same total dose of fungicide, we see a similar pattern to the case with full resistance (figures 4.13 - 4.16). Decreasing the degree of resistance changes the pattern of tactic performance with regard to selection only slightly when resistance is Type 1 (figures 4.13a-d and 4.14a-d). When the resistance is Type 2, however, two new patterns appear in the data. As the degree of resistance decreases, the relationship between the upper bounds and lower bounds of the selection ratios possible for a given level of disease control to that level of control switches from convex to concave (figures 4.13e-h and 4.14e-h). More importantly, the alternation tactics are capable of producing selection ratios lower than mixture for some levels of disease

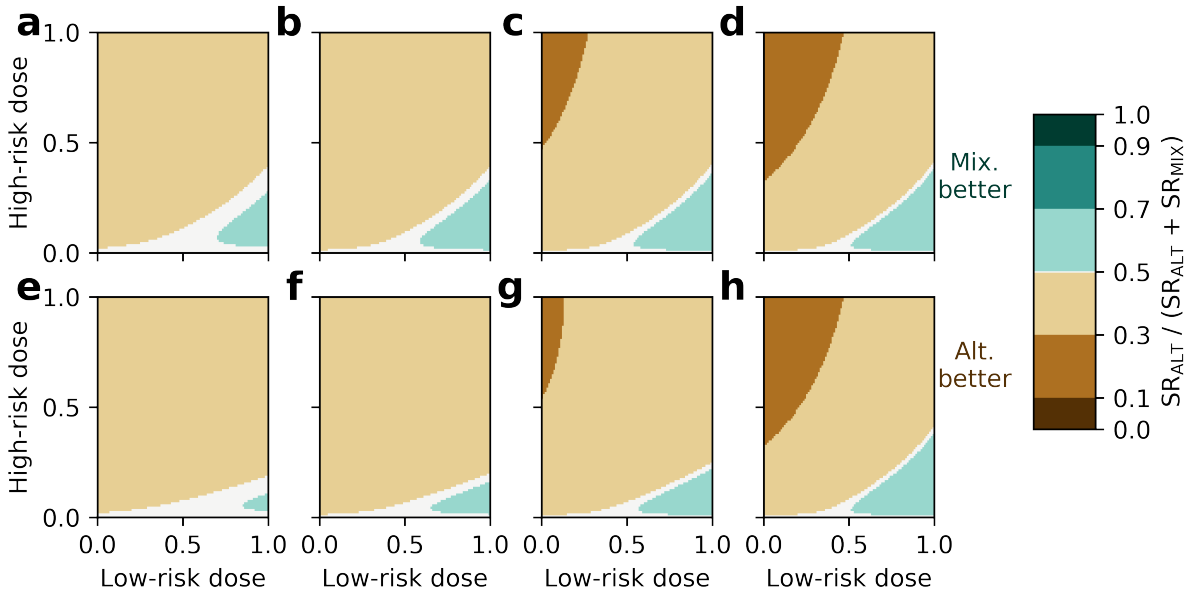

Figure 4.10: Relative performance of mixture and alternation for a variety of dose combinations in the powdery mildew model when resistance to the high-risk fungicide is partial. Predictions shown are based on selection ratio. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1. Note that **d** and **e** are identical and correspond to full resistance.

control when the degree of resistance is low (*e.g.* figure 4.14e when the disease severity is around 1%).

At first glance, it seems that the pattern of tactic yield performance is entirely changed by resistance being partial instead of full (figures 4.15 and 4.16). However, the main cause of the change in pattern is the fact that partial resistance allows infinite effective lives when the dose of high-risk is high. The patterns in yield performance can be seen as the pattern expected for full resistance, with an area of infinite effective life at higher levels of disease control which increases in size as the degree of resistance decreases. As with the selection results, as the degree of resistance decreases, the alternation tactics begin to be able to out-perform mixture for the same level of initial control.

4.6 Discussion

4.6.1 Generality of results

This chapter has shown the generality of some key results of the previous chapter to a range of factors. Mixture performs relatively better than alternation when the applied dose of the high-risk fungicide is low. This is true when the tactics are compared in terms of resistance management or long-term yield. For resistance management purposes, it is also

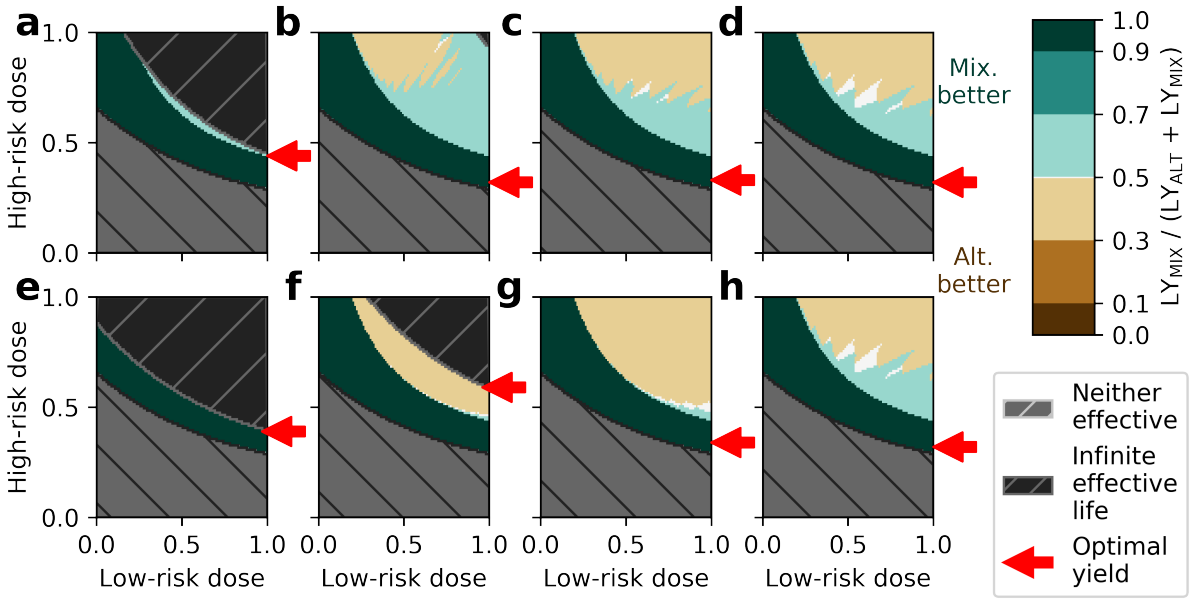

Figure 4.11: Relative performance of mixture and alternation for a variety of dose combinations in the septoria model when resistance to the high-risk fungicide is partial. Predictions shown are based on lifetime yield. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1.

generally true that mixture performs better compared to alternation when the dose of the low-risk is higher. For lifetime yield the relationship between low-risk dose and relative tactic performance is more variable and complex.

The invariance of the results to some key parameters is encouraging, in particular the initial frequency of resistance, infection rate and latent period length. These parameters can vary between times and locations or can even be unknowable in the first instance. It may therefore be that recommendations about whether to use mixture or alternation may be able to be made relatively generally, rather than on a very specific basis. By relatively general we mean that the recommendation can be made on the scale of a particular pathosystem, rather than a particular field. In addition this insensitivity suggests that the uncertainty in the estimation of these parameters for use in our models may be acceptable, as the qualitative results do not depend on their exact value. Lastly this shows that although the difference between tactics may have been small at times in the previous chapter, these differences may be increased by altering the value of these parameters without changing the qualitative ordering of tactic performance.

In all but two cases the overall optimal tactic for lifetime yield was to apply a mixture of as much of low-risk as possible, and only slightly more high-risk than required for initial control. When resistance was Type 2 (affecting curvature of the dose-response curve) and resistance was weak, it was superior to use alternation with a higher dose of the high-risk fungicide. When there was also resistance to the partner fungicide it was still optimal to use

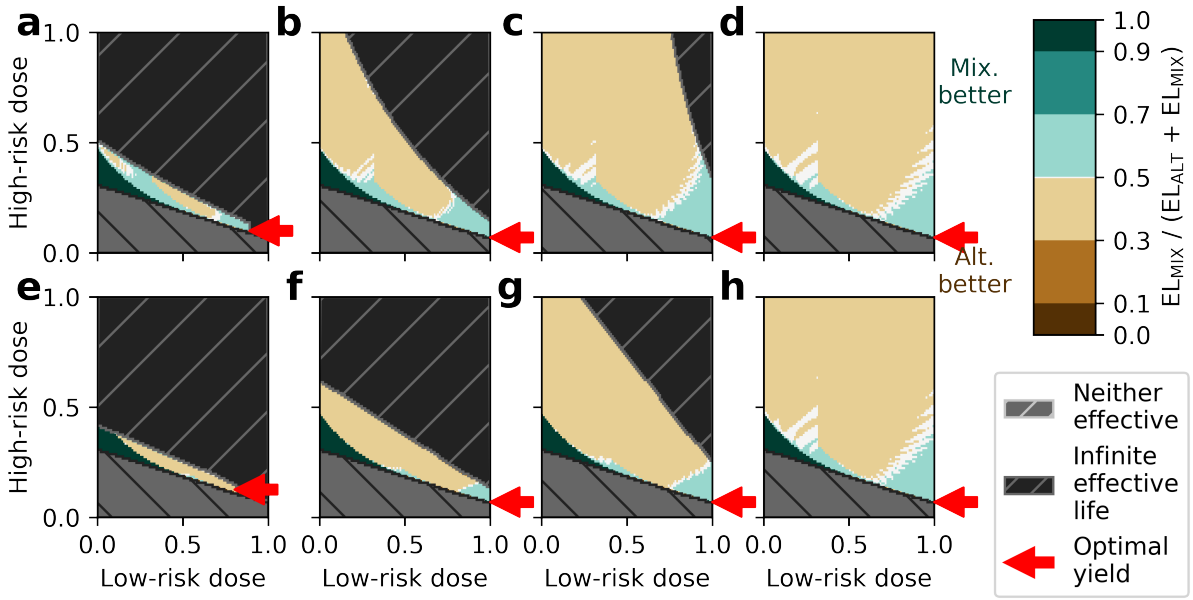

Figure 4.12: Relative performance of mixture and alternation for a variety of dose combinations in the powdery mildew model when resistance to the high-risk fungicide is partial. Predictions shown are based on effective life. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1. Again **d** and **e** are identical and correspond to full resistance.

mixture but with a reduced dose of the partner fungicide.

The overall superior performance of mixture in the presented models is in large part due to the effect of dose-splitting, and so it is perhaps unsurprising the many of the factors investigated did not lead to mixture performing overall worse than alternation. Dose-splitting of the low-risk fungicide means that mixture can produce better disease control even without the use of the high-risk fungicide. This means that mixture can use lower doses of the high-risk than alternation, and thus impose lesser selective pressures, whilst still maintaining disease control. In addition, over the time period when the high-risk dose is applied in mixture the growth rate of the resistant strain is suppressed, reducing selection. Since nothing in the tests presented in this chapter affected this mechanism, it is perhaps unsurprising that many of the keys results remained unchanged.

4.6.2 Importance of dose-response convergence

The only situation in which alternation proved optimal for lifetime yield was with weak Type 2 resistance. This is due to the convergence of the dose-response curves for the resistant and sensitive strains at higher doses. As seen in chapter 3, alternation tends to perform better than mixture at higher doses of the high-risk. If fungicide dose-response curves do not converge within the legal range of doses, then the optimal tactic of mixture of low doses

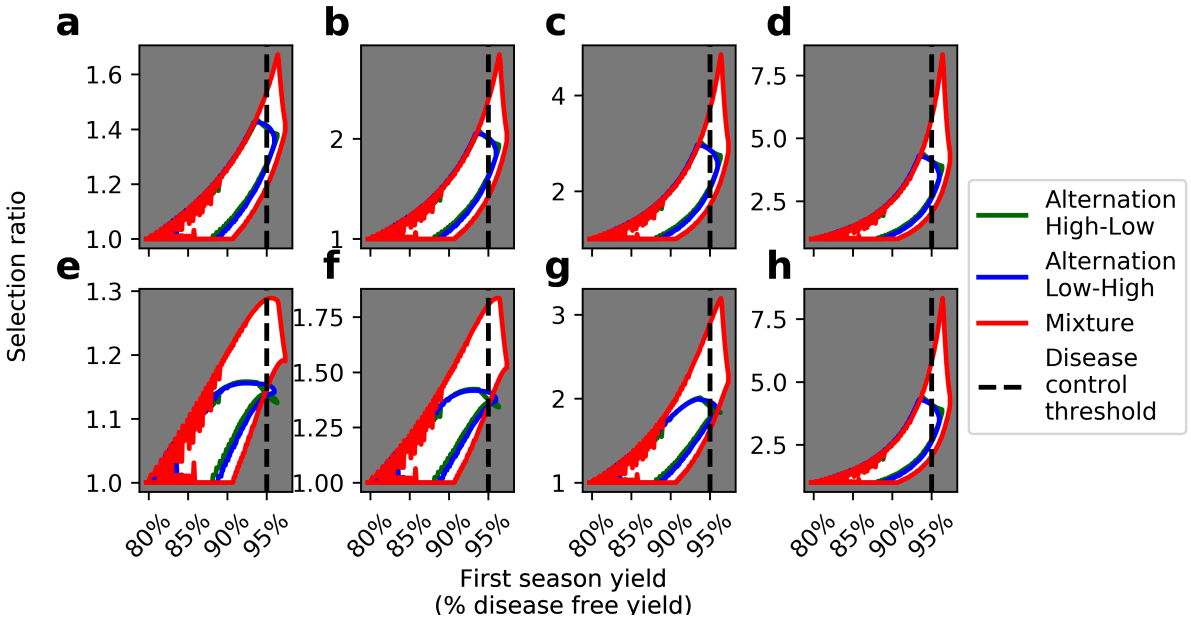

Figure 4.13: Comparison of mixture and alternation tactics in the septoria model when resistance is partial, taking into account the level of disease control they provide. Tactic performance in terms of selection ratio is presented; the polygons show the range of values that can be produced by each tactic. The ”jaggedness” of these images compared to the full resistance images before is simplfy an artefact due to these results having been generated at a lesser number of doses. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1. The simulations were run for a maximum of 200 seasons, and so a value of 200 represents an infinite effective life.

of high-risk and high doses of low-risk would be a practical method for managing resistance in the field. On the one hand, the very fact that resistance is generally reported in terms of a resistance factor suggests that partial resistance is closer to biological reality than full. However, there is strong evidence that reduced dose rather than increased reduces selection and so it is unlikely that dose-response curves converge within the legal range of doses in the majority of cases (van den Bosch *et al.*, 2011). In addition convergence of dose-response curves within the legal dose range implies that the fungicide has similar effects on the resistant and sensitive strains, in which case resistance is unlikely to pose a significant threat to yield.

4.6.3 Insensitivity to epidemiological parameters

The relative performance of the two tactics for resistance management was very insensitive to the value of two key epidemiological parameters, the infection rate and the length of the latent period. This is particularly interesting as these parameters have been identified in the previous literature as being important determinants of which tactic is preferable

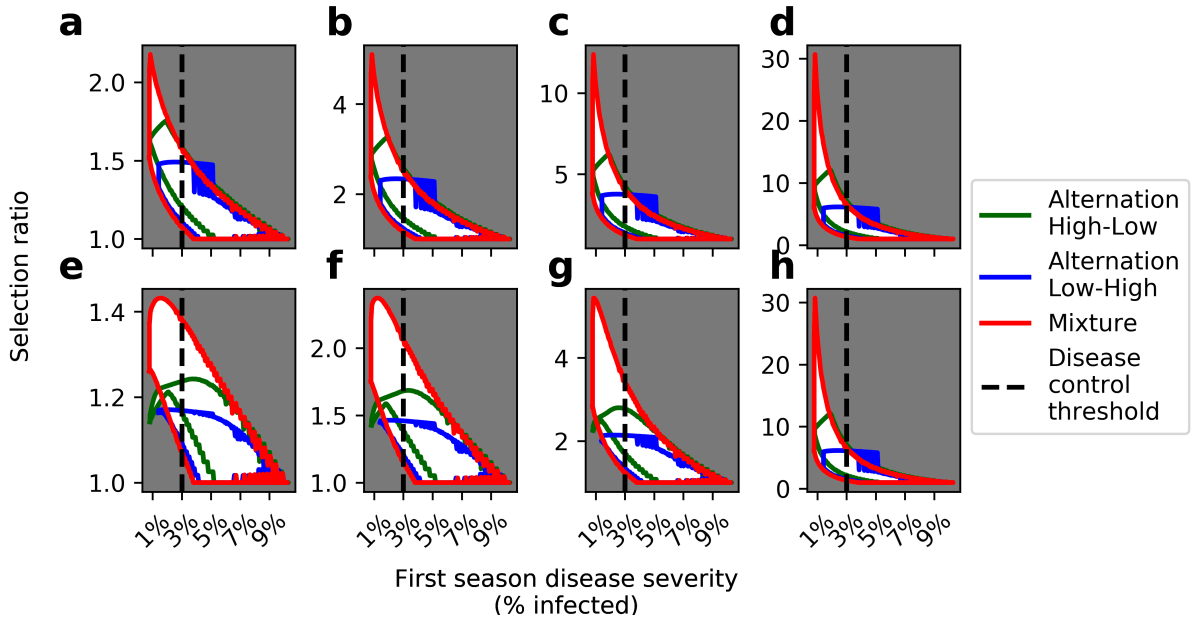

Figure 4.14: Comparison of mixture and alternation tactics in the powdery mildew model when resistance is partial, taking into account the level of disease control they provide. Tactic performance in terms of selection ratio is presented; the polygons show the range of values that can be produced by each tactic. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1. The simulations were run for a maximum of 200 seasons, and so a value of 200 represents an infinite effective life.

(Josepovits & Dobrovolszky, 1985; Skylakakis, 1981). In each of these cases the underlying model of fungicide effect was different to our own. In one case (Josepovits & Dobrovolszky, 1985) the model had an instantaneous reduction in population size along with the reduction in population growth rate, with the strength of either effect varying between mixture and alternation. The other (Skylakakis, 1981) modelled independence of effect of the two fungicides as multiplying their effects on the basic infection rate *sensu* van der Plank (van der Plank, 1963). These differences in the underlying models may explain the variation in sensitivity.

The general result of mixture performing relatively better at lower doses of the high-risk fungicide for lifetime yield, and the optimal tactic being a mixture of as much low-risk fungicide as possible and just a little more high-risk than required for initial control, was also insensitive to the value of these parameters. However the exact nature of the optimal tactic of course depends on these parameters, as their values control the minimal dose of high-risk fungicide needed for control.

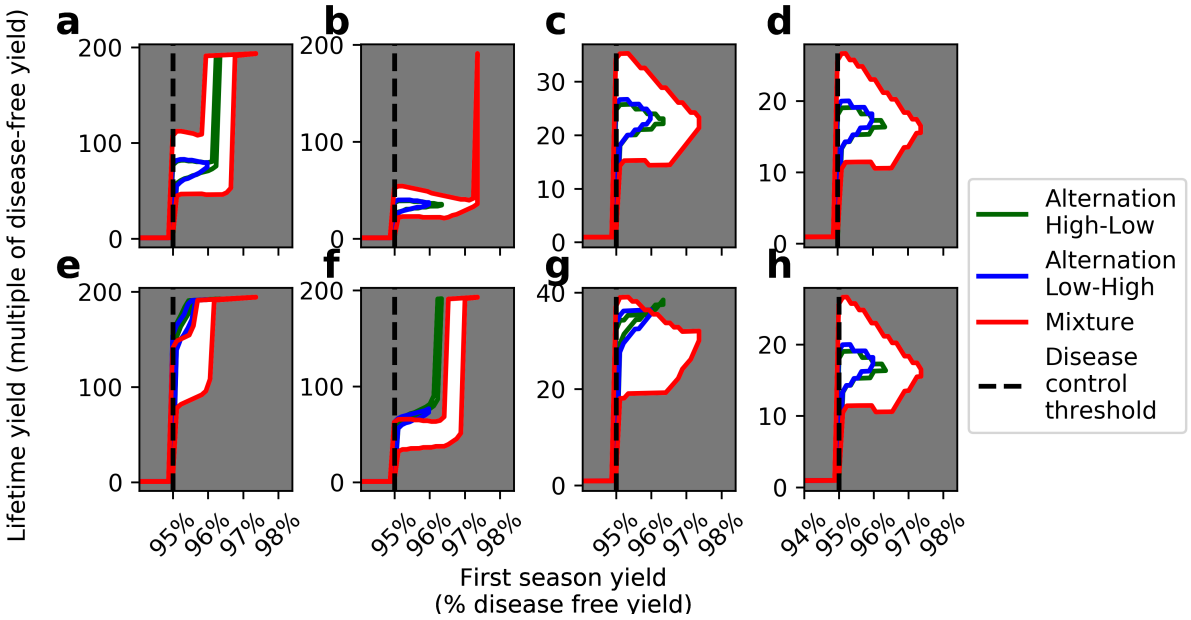

Figure 4.15: Comparison of mixture and alternation tactics in the septoria model when resistance is partial, taking into account the level of disease control they provide. Tactic performance in terms of lifetime yield is presented; the polygons show the range of values that can be produced by each tactic. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1.

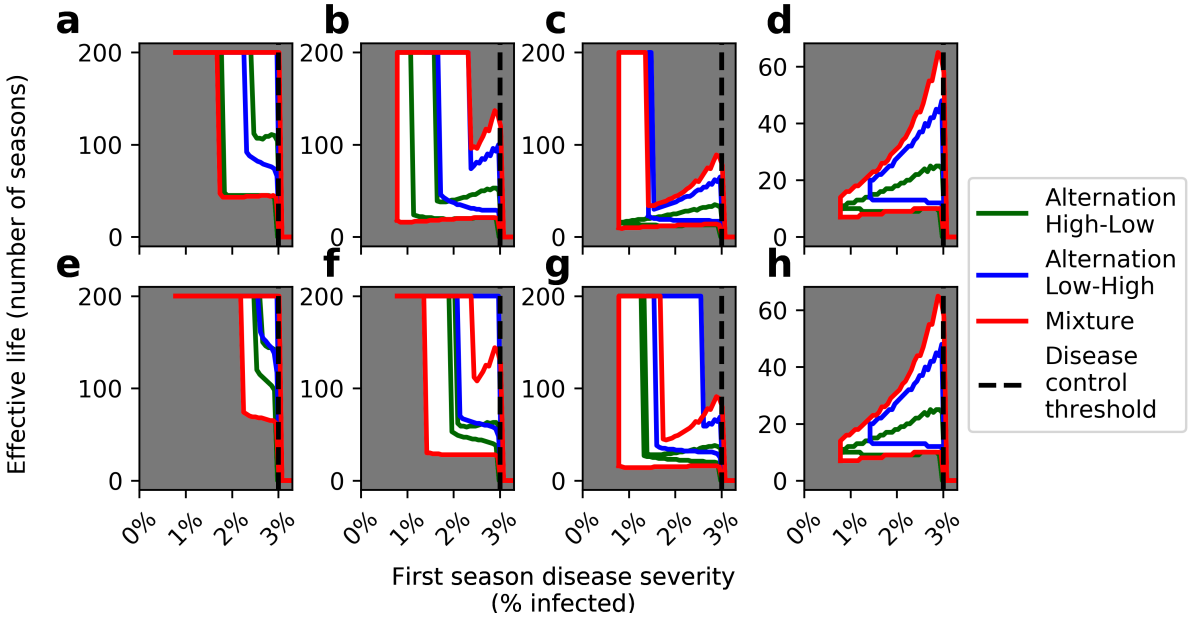

Figure 4.16: Comparison of mixture and alternation tactics in the powdery mildew model when resistance is partial, taking into account the level of disease control they provide. Tactic performance in terms of effective life is presented; the polygons show the range of values that can be produced by each tactic. The top row of panels (**a-d**) show results when resistance affects the maximum effect of the fungicide (Type 1) and the bottom row (**e-h**) when resistance affects the curvature parameter (Type 2). Each column shows a different degree of resistance: **a & e)** r = 0.25, **b & e)** r = 0.5, **c & f)** r = 0.75, and **d & h)** r = 1.

Chapter 4 Summary

- The robustness of the main conclusions of chapter 3 were tested to a number of different factors: parameter values for the pathogen lifecycle and fungicide dose-response curves, the pathosystem modelled, the presence of resistance to the mixing partner and resistance being partial rather than full.
- In all cases alternation tended to perform better at higher doses of the high-risk for resistance management, and mixture at higher doses of the low-risk. For yield purposes mixture tended to perform better at lower doses of either fungicide.
- Only the presence of Type 2 partial resistance (affecting the curvature of the dose-response curve) combined with a low degree of resistance lead to alternation being overall superior for lifetime yield.
- Only adding a strain resistant to the partner fungicide led to a case in which applying as much of the partner fungicide as possible was not optimal.

5

Model structure and the governing principles

> *I'm simply saying that life, uh. . . finds a way.*
>
> Dr Ian Malcolm, *Jurassic Park*, 1993

5.1 Introduction

As described in the introduction (section 1.7), there are simple governing principles for predicting the effect of a fungicide application tactic on resistance evolution. Briefly, the selection coefficient is the measure of the strength of selection for resistance and is given by the difference in per capita growth rates between the resistant sensitive strains (equation 1.1.7),

$$s(t) = \frac{\mathrm{d}N_R(t)}{\mathrm{d}t} N_R(t)^{-1} - \frac{\mathrm{d}N_S(t)}{\mathrm{d}t} N_S(t)^{-1}.$$

where $N_R(t)$ and $N_S(t)$ are the sizes of the fungicide-resistant and -sensitive population respectively. The time integral of the selection coefficient is a quantity we term the cumulative selection coefficient, and gives the total amount of selection for resistance over a given time period (equation 1.1),

$$\sigma = \int_0^T s(t)\,\mathrm{d}t\,.$$

The governing principles state that by examining how a fungicide application tactic impacts the cumulative selection coefficient, we can predict its effect on selection.

In the previous chapters we used the governing principles to explain a number of our results, with the core explanation being the trade-off between dose-splitting and suppression of the resistant strain by the low-risk mixing partner. In this chapter we will formalise the nature of this trade-off, and examine to what extent it explains the previous results. It is worth noting here that the governing principles do not concern disease control or yield, but we will occasionally touch on these during this chapter.

5.2 A simple model based on exponential growth

5.2.1 The model

The models described in chapter 2 are by design complex, as they must be able to recreate the complicated dynamics of real pathosystems. However, this complexity means that analysis becomes more involved and determining the generality of any given result becomes difficult. Therefore to provide a simple framework within which to base our investigation, we begin by introducing a much simpler model. This model is similar to the early models used in the fungicide modelling literature (Delp, 1980; Kable & Jeffery, 1980; Skylakakis, 1981), but with a modern consideration of the fungicide dose-response curve (Hobbelen *et al.*, 2011b). As before we assume there to be two pathogen strains, one fully resistant to the high-risk fungicide (I_R) and one fully sensitive (I_S). We assume that these strains grow exponentially with no latent period such that,

$$\frac{\mathrm{d}I_R}{\mathrm{d}t} = \beta_R(C_L)I_R \tag{5.1}$$

$$\frac{\mathrm{d}I_S}{\mathrm{d}t} = \beta_S(C_L, C_H)I_S\,, \tag{5.2}$$

where $\beta_R(C_L)$ and $\beta_S(C_H, C_L)$ are growth rates dependent on the dose of the high-risk (C_H) and low-risk fungicide (C_L). These growth rates are set by dose-response curves of the same form used in the previous chapters,

$$\beta_R(C_L) = \beta\left(1 - \epsilon_L\left(C_L\right)\right) \tag{5.3}$$

$$\beta_S(C_L, C_H) = \beta\left(1 - \epsilon_H\left(C_H\right)\right)\left(1 - \epsilon_L\left(C_L\right)\right)\,, \tag{5.4}$$

where β is the baseline infection rate in the absence of chemical control, and ϵ_H and ϵ_L are the effects of the fungicides at given doses (see section 2.2.2).

We further assume that the concentration of the fungicides is piece-wise constant, rather than decaying exponentially. Fungicides are applied at a given dose and remain at that concentration for a fixed time until they are instantaneously removed. As before we will assume that there are twice the number of sprays of each fungicide under mixture as under alternation, and at half the dose. In addition we assume that the sprays are independent; there is no overlap of fungicides applied at one spray with those applied at another.

5.2.2 The cumulative selection coefficient

In order to determine the impact of the tactics on fungicide resistance evolution we calculate the cumulative selection coefficient under each. For half of the sprays under alternation there is no selection as no high-risk fungicide is applied, and for the other half the selection

coefficient (when there is no low-risk fungicide) is,

$$\begin{aligned} s_{\text{ALT}} &= \frac{\mathrm{d}I_R}{\mathrm{d}t} I_R^{-1} - \frac{\mathrm{d}I_S}{\mathrm{d}t} I_S^{-1} \\ &= \beta - \beta(1 - \epsilon_H(C_H)) \\ &= \beta \epsilon_H(C_H)\,. \end{aligned} \tag{5.5}$$

Note that the simplicity of this model means that there is no difference between the two alternation tactics used in the previous chapter, and so they are treated as one here.

Under mixture every spray imposes selection according to,

$$\begin{aligned} s_{\text{MIX}} &= \beta\left(1 - \epsilon_L\left(\frac{C_L}{2}\right)\right) - \beta\left(1 - \epsilon_L\left(\frac{C_L}{2}\right)\right)\left(1 - \epsilon_H\left(\frac{C_H}{2}\right)\right) \\ &= \beta\left(1 - \epsilon_L\left(\frac{C_L}{2}\right)\right)\epsilon_H\left(\frac{C_H}{2}\right)\,. \end{aligned} \tag{5.6}$$

If we assume that the time of exposure is the same for each spray, then if alternation imposes selection for T units of time, mixture imposes selection for $2T$. Since the concentration of fungicides is constant integration of equations 5.5 and 5.6 with respect to these times of exposure is very simple, and leads to the cumulative selection coefficient for each tactic,

$$\begin{aligned} \sigma_{\text{ALT}} &= \int_0^T s_{\text{ALT}}\,\mathrm{d}t \\ &= \beta \epsilon_H(C_H) T \end{aligned} \tag{5.7}$$

$$\begin{aligned} \sigma_{\text{MIX}} &= \int_0^{2T} s_{\text{MIX}}\,\mathrm{d}t \\ &= 2\beta\left(1 - \epsilon_L\left(\frac{C_L}{2}\right)\right)\epsilon_H\left(\frac{C_H}{2}\right) T\,. \end{aligned} \tag{5.8}$$

By looking at the effect of changing C_H and C_L on the value of the cumulative selection coefficients we see that as expected increasing the dose of the high-risk increases selection under both tactics, but increasing the dose of the low-risk only decreases selection under mixture.

To investigate which tactic leads to better resistance management we take the ratio of the cumulative selection coefficients,

$$z = \frac{\sigma_{\text{ALT}}}{\sigma_{\text{MIX}}} \tag{5.9}$$

$$= \frac{\epsilon_H(C_H)}{2\left(1 - \epsilon_L\left(\frac{C_L}{2}\right)\right)\epsilon_H\left(\frac{C_H}{2}\right)}\,. \tag{5.10}$$

If $z > 1$ then mixture is better, if $z < 1$ then alternation, and if $z = 1$ they perform equally well. It is encouraging that both the time of exposure and the infection rate parameter cancel out of this comparison, as both are arbitrary. Thus, the relative performance of mixture and

alternation is decided entirely by the parameters controlling the dose-response curves of the fungicides. Expanding out the effect terms,

$$\begin{aligned} z &= \frac{\omega_H(1-\mathrm{e}^{-\theta_H C_H})}{2(1-\omega_L(1-\mathrm{e}^{-\theta_L \frac{C_L}{2}}))\omega_H(1-\mathrm{e}^{-\theta_H \frac{C_H}{2}})} \\ &= \frac{1+H}{2\left(1-\omega_L\left(1-L\right)\right)} \;\Big|\; C_H > 0\,. \end{aligned} \tag{5.11}$$

where

$$H = \mathrm{e}^{-\theta_H \frac{C_H}{2}} \tag{5.12}$$

$$L = \mathrm{e}^{-\theta_L \frac{C_L}{2}}\,. \tag{5.13}$$

Although z (equation 5.11) is undefined for $C_H = 0$, clearly $\sigma_{\mathrm{ALT}} = \sigma_{\mathrm{MIX}}$ in this case and so the strategies perform equally well. Notably the maximum effect of the high-risk fungicide (ω_H) does not appear in equation 5.11. Increasing the effect of the low-risk will tend to favour mixture, and of the high-risk (by increasing θ_H) will favour alternation.

5.2.3 Patterns in dose-space

In the previous chapters, for resistance management we observed that the tactics performed identically when no high-risk was applied, then for any given low-risk dose as the high-risk dose was increased first mixture performed better, then the tactics performed identically and finally alternation out-performed mixture. We can find the the points (C_L^*, C_H^*) where the tactics perform equally well by equating the right-hand side of 5.11 to 1,

$$1 = \frac{1+\mathrm{e}^{-\theta_H C_H^*}}{2\left(1-\omega_L\left(1-\mathrm{e}^{\frac{-\theta_L C_L^*}{2}}\right)\right)}\,, \tag{5.14}$$

which leads to

$$C_H^* = \frac{-2}{\theta_H}\ln\left(1-2\epsilon_L\left(\frac{C_L^*}{2}\right)\right)\,. \tag{5.15}$$

We shall refer to this relationship as the boundary curve from now on. Our simple model agrees with the observations of the previous chapters that the tactics should perform equally well with no high-risk. If the effect of the low-risk under mixture is low enough ($\epsilon_L(\frac{C_L^*}{2}) < \frac{1}{2}$) then there will also be a higher dose of the high-risk which leads to equal performance between the tactics. This same boundary was previously identified in Shaw (1989b). Whether mixture can out-perform alternation at high doses of high-risk is therefore set by the value of ω_L, as this controls whether the effect of the low-risk can ever exceed $\frac{1}{2}$. The mechanism behind this is that as $C_H \to \infty$, $\epsilon_H(\frac{C_H}{2}) \to \epsilon_H(C_H)$ and so the cost of dose-splitting under

mixture increases. Whether the cost of dose-splitting can be overcome by suppression of the resistant strain by the mixing partner therefore depends on whether the effect of the low-risk can make up for the near-doubling of the high-risk effect under mixture compared to alternation, hence the critical value of $\frac{1}{2}$. With this intuition, it is clear that we can generalise this result to say that if the alternation dose is split n times under mixture, the low-risk must exert an effect of at least $\frac{1}{n}$ at each of those sprays to compensate.

Next we take the derivative of the boundary curve with respect to the low-risk dose,

$$\frac{\mathrm{d}C_H^*}{\mathrm{d}C_L^*} = \frac{2\omega_L\theta_L \mathrm{e}^{-\theta_L \frac{C_L^*}{2}}}{\theta_H\left(1 - 2\epsilon_L\left(\frac{C_L^*}{2}\right)\right)}\,. \tag{5.16}$$

This derivative is strictly positive unless $\epsilon_L(\frac{C_L^*}{2}) \geq \frac{1}{2}$ in which case it is undefined or negative, but the boundary function itself is also undefined for this condition. Therefore the boundary is monotonically increasing if it exists.

We take the derivative again,

$$\frac{\mathrm{d}^2C_H^*}{\mathrm{d}C_L^{*2}} = \frac{\omega_L(2\omega_L - 1)\theta_L^2 \mathrm{e}^{\theta_L \frac{C_L^*}{2}}}{\theta_H\left(2\epsilon_L\left(\frac{C_L^*}{2}\right) - \mathrm{e}^{\theta_L \frac{C_L^*}{2}}\right)^2}\,. \tag{5.17}$$

The sign of 5.17 is controlled entirely by the maximum effect of the low-risk: if greater then $\frac{1}{2}$ then positive, if lesser positive and if equal zero. This implies that the boundary curve is in fact straight if $\omega_L = \frac{1}{2}$, concave if lesser and convex if greater. This matches the shape of the curves observed in the previous chapter where for the powdery mildew model the low-risk (sulphur) has a maximum effect of 1, and in the septoria model the low-risk (chlorothalonil) has a maximum effect of 0.48. In both models we always saw a strictly positive high-risk dose, as well as zero dose, that led to equal performance of both tactics for resistance management; this is just due to truncation of the dose axes into the area of legal dose-space.

Our conclusions about the effect of each parameter included in the simple model held up more or less in the full model of septoria (figure 4.1). The maximum effect of the high-risk had relatively little effect on the comparison of tactics as predicted by the simple model, only scaling the differences between tactics. The infection rate and initial frequency of resistance also had little effect as expected. Increasing the curvature parameter of the high-risk favoured alternation as predicted, and increasing the maximum effect of the low-risk favoured mixture. The effect of the low-risk curvature however was not as expected. The simple model predicts that increasing this parameter should favour mixture, however in the complex model this was only true up to a point, and further increases favoured alternation. This is because the complex model violates the assumption in the simple model that the fungicides from different sprays do not overlap.

5.2.4 Partial resistance

This simple model can readily adapted to consider partial resistance, in order to explore the effects seen in the previous chapter (see section 4.5). We can consider partial resistance by multiplying either the maximum effect (Type 1 resistance) or curvature parameter (Type 2) of the high-risk fungicide dose-response curve for the resistant strain by $(1 - r)$, where r is the degree of resistance. For Type 1 resistance this will clearly have no effect on relative tactic performance in the simple model as the maximum effect of the high-risk has no bearing either. This fits with the results of the models of the previous chapter, where reducing the degree of Type 1 resistance decreased the difference between tactics but did not change which performed better for a given pair of doses.

Type 2 resistance however leads to a different form for the ratio of cumulative selection coefficients between tactics. Following the same procedure as above we get,

$$\sigma_{\mathrm{ALT}} = \beta\omega_H \left(1 - \mathrm{e}^{-\theta_H(1-r)C_H}\right) T \tag{5.18}$$

$$\sigma_{\mathrm{MIX}} = 2\beta \left(1 - \omega_L \left(1 - \mathrm{e}^{-\theta_L \frac{C_L}{2}}\right)\right) \omega_H \left(1 - \mathrm{e}^{-\theta_H(1-r)\frac{C_H}{2}}\right) T \tag{5.19}$$

$$z = \frac{1 + H^{1-r}}{2(1 - \omega_L(1 - L))}\,, \tag{5.20}$$

If we take the derivative with respect to r,

$$\frac{\mathrm{d}z}{\mathrm{d}r} = \frac{(1 - r)H^{-r}}{2(1 - \omega_L(1 - L))}\,, \tag{5.21}$$

which is always positive and so a greater degree of resistance will favour mixture. This too holds with the results seen in the previous chapter.

5.3 Models of intermediate complexity

5.3.1 Distinguishing features

There are 5 features in the FiveLeaf model that are not in the simple model.

- **Fungicide decay** In the simple model fungicides remain at fixed concentration, whereas they decay exponentially over time in the FiveLeaf model.
- **Host-limited infection** In the FiveLeaf model the amount of remaining susceptible host tissue affects the rate of infection, whereas in the simple model the rate of infection is, apart from the effect of fungicides, constant.
- **Latent infection** In the FiveLeaf model tissue first enters a latently-infected class before becoming infectious. In the simple model infected tissue is infectious straight away.

- **Phenology** There are a couple of features in the FiveLeaf model related to intra-season timing which we will refer to under the blanket term ”phenology”. These features are the senescence of living tissue and the initiation of the seasonal epidemic by primary inoculum.
- **Seasonality** The FiveLeaf model explicitly considers the difference between consecutive seasons. The simple model has no concept of a season.

The final feature is unimportant as the cumulative selection coefficient is independent of the size of the pathogen populations when growth is exponential, so a single season or multiple seasons with periodic resetting of the population sizes will lead to the same predictions in the simple model. For ease of comparison, we will treat the simple exponential model as an inter-season model with assumed discrete state changes between seasons matching those of the more complex models from now on.

5.3.2 Constructing a range of sub-models

By including or excluding each of the four interesting features (fungicide decay, host-limited infection, latent infection and phenology) we can construct 16 different sub-models of varying complexity for each pathosystem ranging from the simple exponential model to the full model with all features included. The simple exponential model gives a result in terms of the trade-off between dose-splitting and suppression of the resistant strain by the mixing partner alone. By examining to what extent each of these models differs from the result of the simple model, we can isolate the impact of different features on this trade-off and also potentially identify other mechanisms that lead to a difference in performance between mixture and alternation.

For the majority of parameters, the same values can be used between models. However the infection rate parameter (β) is essentially a free parameter, which summarises the rates of a large number of biological processes. Therefore we refit the infection rate parameter for each model. This is done by taking the full model as the source of truth for the system. Then the Nelder-Mead simplex algorithm is used to choose a value of the infection parameter in the simplified sub-model that gives the smallest least-squares difference between the daily amount of infectious tissue in that sub-model and the full model. As the infection severity responds to different degrees to a given dose of fungicide in each of the sub-models, the minimisation objective function uses squared differences across multiple doses (0.25, 0.5, 0.75, 1) of each fungicide and each application tactic. The best-fitting values of the infection rate for each sub-model are listed in appendix A.1.2.

5.3.3 Comparing predictions with the cumulative selection coefficient and selection ratio

In the previous chapter we used the selection ratio to quantify the degree of resistance development, whereas thus far in this chapter we have used the cumulative selection coefficient. The cumulative selection coefficient is a useful metric for the simple model as an analytical form can be produced, but for the more complicated sub-models this is either not possible or more complicated and so we resort to the selection ratio.

The cumulative selection coefficient is the time integral of the difference in per capita growth rates between the resistant and sensitive strains (equation 1.1). The selection ratio is the ratio of resistance frequencies at the end and beginning of the first season, and as such the time period of interest for the comparison of metrics is that first season. We note that the cumulative selection coefficient may vary slightly between seasons in the more complex models, as the resistance frequency affects the overall level of disease control and thus the growth rates of the pathogen strains. Although different, the two metrics are related by a strictly increasing function and so the qualitative predictions of either can be directly compared (box 5.1).

5.3.4 Adding fungicide decay

Of the features included in the sub-models, fungicide decay requires special consideration as it changes the relationship between fungicide dose and the total effect on the pathogen. The total effect on the pathogen can be described by the time integral of the effect. Under fixed dose (C) this gives,

$$\int_0^T \epsilon(C)\,\mathrm{d}t = \omega(1-\mathrm{e}^{-\theta C})T\,, \tag{5.22}$$

and under decaying dose,

$$\int_0^T \epsilon(C)\,\mathrm{d}t = \omega\left(T - \int_0^T \mathrm{e}^{-\theta C \mathrm{e}^{-\delta t}}\,\mathrm{d}t\right)\,, \tag{5.23}$$

which has no analytical solution. These two integrals have different responses to dose and time of exposure. As the time of exposure increases, the integral with constant dose increases and is unbounded, whereas the integral with decay increases but is bounded. As the dose is increased both integrals increase, but at different rates.

As the same dose under each treatment of fungicide persistance leads to differing total effects on the pathogen over time, it would be useful to be able to find a transformation between doses under each model that lead to the same effect. This would help in matching the simpler model to the more complex model, to increase the accuracy of predictions. That is, if the dose in the model with no decay is C_N we look for the matching dose C_D in the

Box 5.1: Relationship between the cumulative selection coefficient and selection ratio

The cumulative selection coefficient over the first season (from time 0 to T) is

$$\sigma = \int_0^T \left(\frac{\mathrm{d}I_R(t)}{\mathrm{d}t} I_R(t)^{-1} - \frac{\mathrm{d}I_S(t)}{\mathrm{d}t} I_S(t)^{-1} \right) \mathrm{d}t \,, \tag{5.1.1}$$

and the selection ratio is

$$\mathrm{SR} = \frac{\phi(T)}{\phi(0)} \,, \tag{5.1.2}$$

where $\phi(t)$ is the frequency of resistance at time t,

$$\phi(t) = \frac{I_R(t)}{I_R(t) + I_S(t)} \,. \tag{5.1.3}$$

Evaluating the integral in 5.1.1,

$$\sigma = \Big[\ln\left(I_R(t)\right) - \ln(I_S(t)) \Big]_0^T = \ln\left(\frac{I_R(T) I_S(0)}{I_R(0) I_S(T)} \right) \,. \tag{5.1.4}$$

Using 5.1.2 and 5.1.4,

$$\begin{aligned} \sigma &= \ln(\mathrm{SR}) - \ln\left(\frac{(I_R(0) + I_S(0)) I_S(T)}{(I_R(T) + I_S(T)) I_S(0)} \right) \\ &= \ln(\mathrm{SR}) - \ln\left(\frac{1 - \phi(T)}{1 - \phi(0)} \right) \,. \end{aligned} \tag{5.1.5}$$

Using 5.1.2 leads to an expression for the selection coefficient in terms of the selection ratio and the constant $\phi(0)$,

$$\begin{aligned} \sigma &= \ln(\mathrm{SR}) - \ln\left(\frac{1 - \phi(0)\mathrm{SR}}{1 - \phi(0)} \right) \\ &= \ln(\mathrm{SR}) + \ln(1 - \phi(0)) - \ln(1 - \phi(0)\mathrm{SR}) \end{aligned} \tag{5.1.6}$$

Taking the derivative with respect to the selection ratio,

$$\frac{\partial \sigma}{\partial(\mathrm{SR})} = \frac{1}{\mathrm{SR}(1 - \phi(0)\mathrm{SR})} = \frac{1}{\mathrm{SR}(1 - \phi(T))} \tag{5.1.7}$$

$$\therefore \phi(T) < 1 \implies \frac{\partial \sigma}{\partial(\mathrm{SR})} > 0 \,. \tag{5.1.8}$$

The cumulative selection coefficient is therefore strictly increasing with the selection ratio as $\phi(T) < 1$. Consequently the order of preference of tactics compared with one metric will be the same as with the other, justifying using the metrics interchangeably.

decaying model where

$$\int_0^{T_N} \epsilon(C_N)\, \mathrm{d}t = \int_0^{T_D} \epsilon(C_D)\, \mathrm{d}t \,, \tag{5.24}$$

where T_N and T_D are the times of exposure in the constant dose and exponential decay model respectively. These times of exposure need not necessarily be the same. A reasonable choice might be to treat the exponential decay model as the more realistic, and assume that in the field fungicide doses decay away to negligible levels over the course of the epidemic, and therefore set the time of exposure in the exponential decay model to be infinite. Using 5.22 and 5.23 in 5.24,

$$C_N = \lim_{T_D \to \infty} \left(\frac{-1}{\theta} \ln \left(1 - \frac{1}{T_N} \left(T_D - \int_0^{T_D} \mathrm{e}^{-\theta C_D \mathrm{e}^{-\delta t}} \right) \right) \right) . \tag{5.25}$$

This transformation leads to a positive value of C_N if and only if

$$0 < \lim_{T_D \to \infty} \left(\frac{1}{T_N} \left(T_D - \int_0^{T_D} \mathrm{e}^{-\theta C_D \mathrm{e}^{-\delta t}} \right) \right) < 1 . \tag{5.26}$$

Clearly therefore the value of T_N controls the mapping between doses in the two models, and in a non-linear way. There is no obvious choice of what value to use for this time of exposure. Furthermore it is not just the integral of the effect that is important, but also how the concentrations of the low-risk and high-risk intersect in time.

Given the difficulty in normalising doses in models without exponential decay, we choose to ignore this and treat doses the same in all models. There is no clear way to improve on this, and so we must simply accept that the results of the models without fungicide decay are not directly compatible with those that include decay. Due to this inherent difference, we will ignore all possible sub-models without fungicide decay apart from the simple exponential model, which we keep since it is a useful starting point.

5.3.5 Tactic performance for resistance management

We first investigate how the prediction of tactic performance in terms of resistance management varies between the models (figure 5.1). The first noticeable feature of these predictions is that they are all strikingly similar, even the simple model without fungicide decay, which might be expected to be very inaccurate for the reasons described above. The shape of the boundary curve matches closely what is predicted by the simple model. The area of dose-space for which mixture out-performs alternation is significantly smaller in the full model compared to the the sub-models with only one or two model features included. Including latent infection has the largest effect on the pattern of tactic performance, after the effect of including fungicide decay. This is perhaps unsurprising as without the latent period the eradicant effect of the high-risk fungicide is entirely ignored. Removing the latent period effectively makes the high-risk fungicide less effective, favouring mixture. Models differing only in the presence of host-limited infection produce near identical results, suggesting that the assumption of exponential growth of pathogen over the period of time when fungicides

are applied may be a reasonable one.

5.4 Tactic performance for lifetime yield

For sub-models including host-limited infection we can also generate a prediction of tactic yield performance (figure 5.2). These predictions are more variable than those based on resistance management alone but the same characteristic pattern is seen in each sub-model as in the full model. For any given dose of low-risk, as the high-risk dose is increased first all tactics fail to give acceptable disease control, then mixture out-performs alternation, and finally alternation out-performs mixture. With the reduced number of sub-models it is more difficult to draw comparisons between model features but yet again it seems as if including latent infection has a large effect on the predictions. For all models the overall optimal tactic was to apply as much low-risk as possible and slightly more high-risk than needed for initial control and to do so under mixture.

The difference between the minimal dose of high-risk needed for control and the optimal dose of high-risk is due to requiring a buffer of disease control. If the applied dose were exactly the minimal dose needed for control for example, then control would necessarily fail in the second season once any amount of selection had occurred. The optimal dose is not necessarily the highest dose that gives the longest effective life and thus has the largest buffer, however. This is because higher doses of the high-risk lead to greater selection and thus can cause more catastrophic failure of control in the final season over which the lifetime yield is calculated. This poorer control in the final season can offset the increased control the higher dose gives in the initial seasons and lead to an overall lower lifetime yield. The optimal dose of high-risk in the simplest model is perhaps surprisingly high, but this just represents that selection is stronger due to the lack of selection-weakening mechanisms in the more complex models.

5.5 A more complex model of host tissue dynamics

5.5.1 The model

There are still a number of aspects of the real pathosystem not considered in the models presented so far. Some of these features are included in the more complex ElevenLeaf model (see section 2.4.3 for full description), which in particular includes following features.

- The individual development of each separate leaf layer.
- Eradicant fungicides only affect lesions in the first half of their latent period,
- Different leaves contribute toward yield production and infectious inoculum in the next season to different degrees.

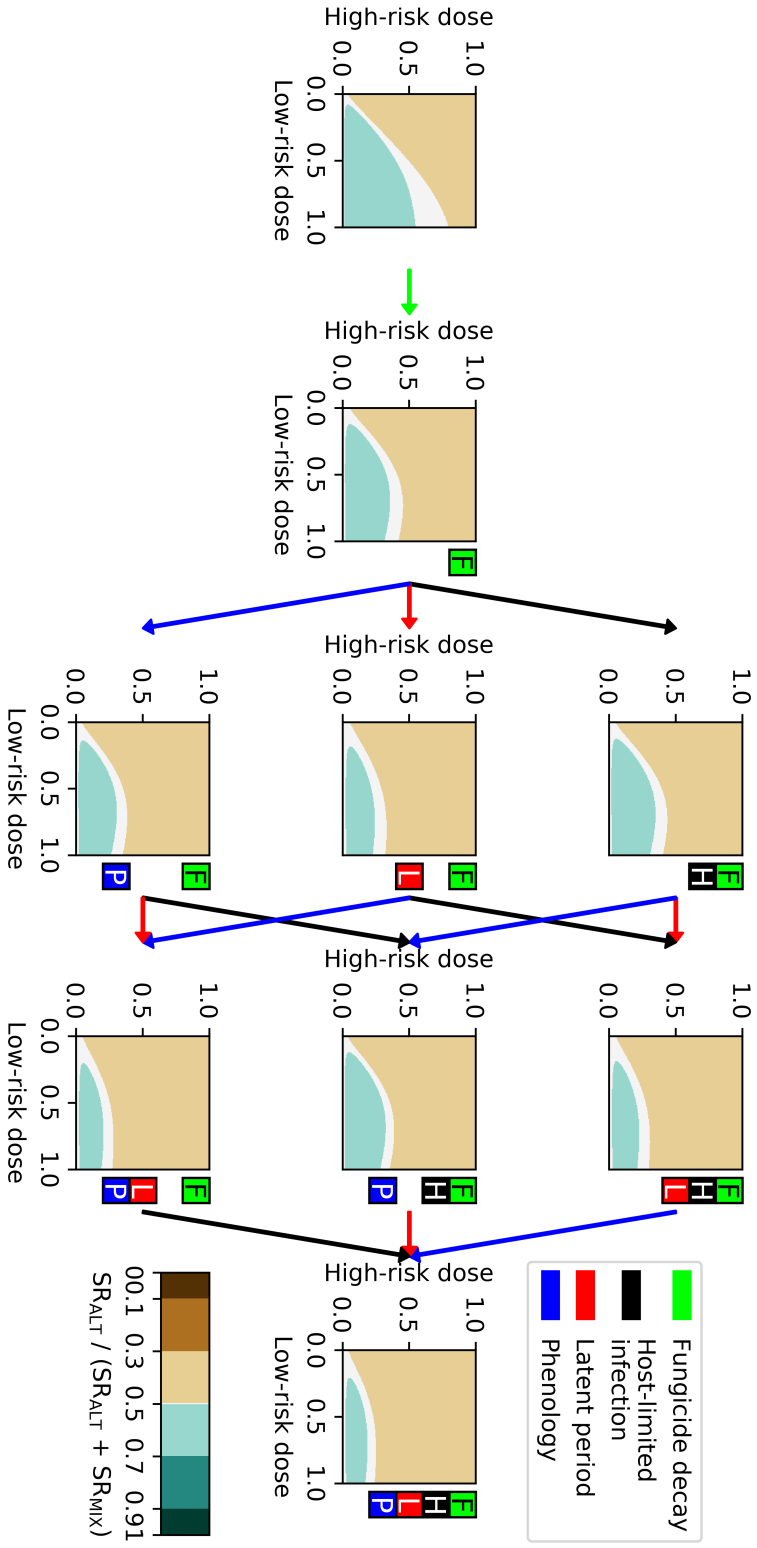

Figure 5.1: Comparison of performance of mixture and alternation for resistance management in a range of models of septoria leaf blotch. The models increase in complexity from left to right. The coloured boxes identify which features are present in the models underlying each prediction. The coloured arrows identify which feature must be added to a model to get the model directly on the right.

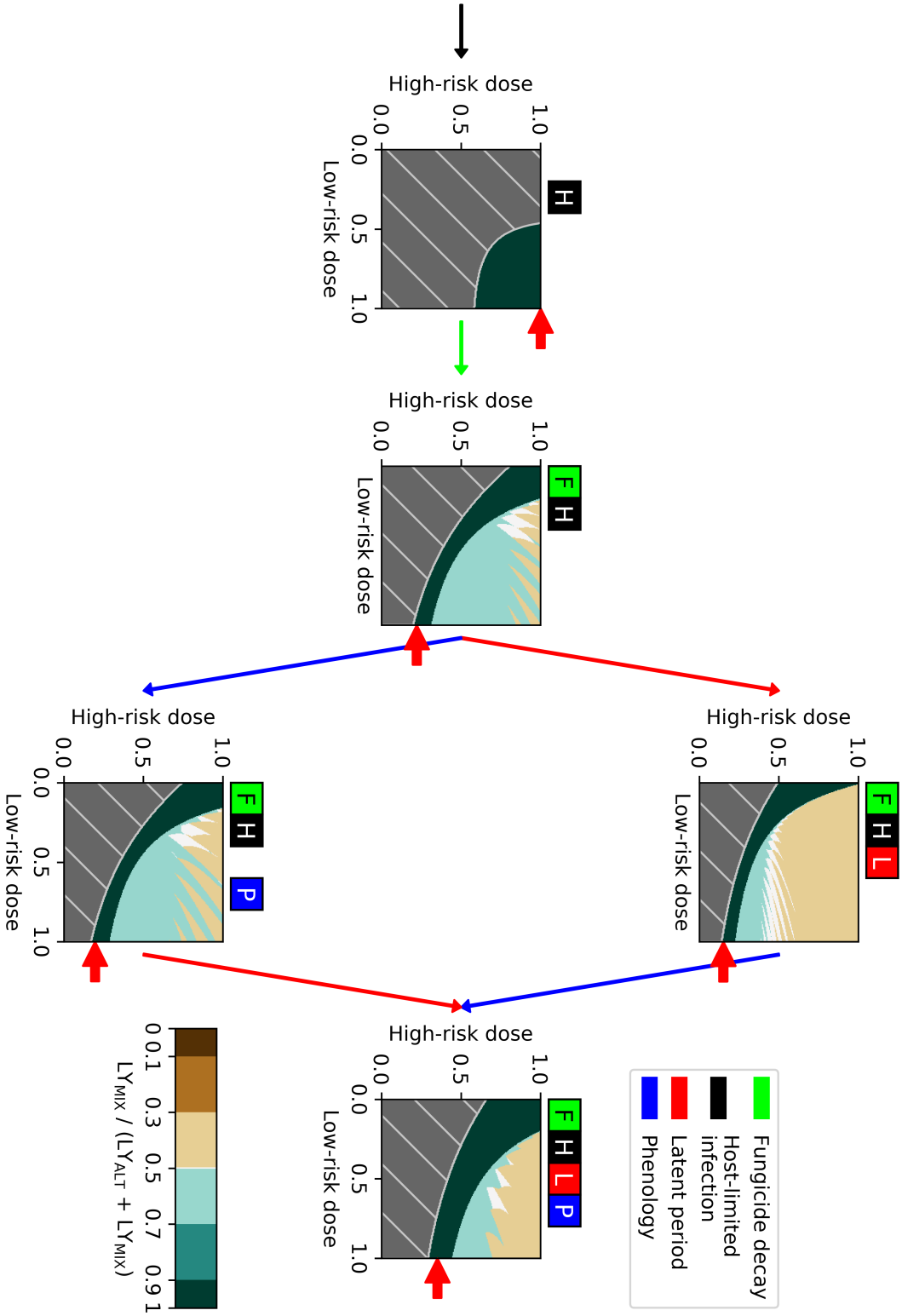

Figure 5.2: Comparison of performance of mixture and alternation for lifetime yield in a range of models of septoria leaf blotch. The models increase in complexity from left to right. The coloured boxes identify which features are present in the models underlying each prediction. The coloured arrows identify which feature must be added to a model on the left to get a model on the right.

We wish to examine whether the findings of our simpler FiveLeaf model match closely those of the more complex ElevenLeaf model. Note that the fungicide parameter values used in the ElevenLeaf model (appendix A.1.3) are distinct to those used in the FiveLeaf despite the fungicides modelled being the same, since the same parameter values lead to different effects in the different models.

5.5.2 Fixed total dose

The first comparison to make is to compare mixture and alternation at fixed total dose (figure 5.3). A very similar pattern for the selection pressure imposed by the tactics is seen as in the other models, where alternation imposes less selection at higher doses of the high-risk and mixture at higher doses of the low-risk. As before, the overall optimal tactic for lifetime yield is to apply a mixture of as much low-risk as possible and slightly more high-risk than required for initial disease control. There is a notable step-change in the trade-off between mixture and alternation for resistance management at a high-risk dose of around 0.4. This is because when the high-risk dose is lower than around 0.4, the Alternation Low-High tactic imposes less selection and when above the High-Low (figure 5.4).

We can visualise the performance of the tactics in more detail by plotting out metrics for each tactic as a response to the high-risk dose when the low-risk dose is fixed at maximum (figure 5.5). For all doses of the high-risk mixture provides greater disease control initially, with this becoming more apparent at greater doses. At low dose of the high-risk, mixture imposes less selection than either alternation tactic, and Alternation High-Low imposes more selection than Alternation Low-High. As the dose is increased all three tactics impose more selection but the increase in selection is smallest for Alternation High-Low, and largest for mixture with Alternation Low-High being intermediate. Note that the dose at which Alternation High-Low imposes less selection than Alternation Low-High is largely invariant to the dose of the low-risk (from figure 5.4) whilst the dose at which either of the alternation tactics imposes less selection than mixture is heavily dependent on the low-risk dose (from figure 5.3).

As in the simpler model, for any given level of disease control, mixture is capable of producing lesser selection ratios and greater effective lives (figure 5.6). However compared to the simpler model the performance of the worst mixture is increased. In the FiveLeaf model mixture could give much better but also much worse results for any level of control, whereas in the ElevenLeaf model the difference between the worst mixture and the worst alternation is much smaller.

5.5.3 A counterintuitive effect of the low-risk fungicide

In the simpler models increasing the dose of the low-risk would at worst have no effect on selection, and would tend to decrease selection by suppressing the growth of the resistant strain. This is not necessarily the case in this model, for example when the dose of the high-

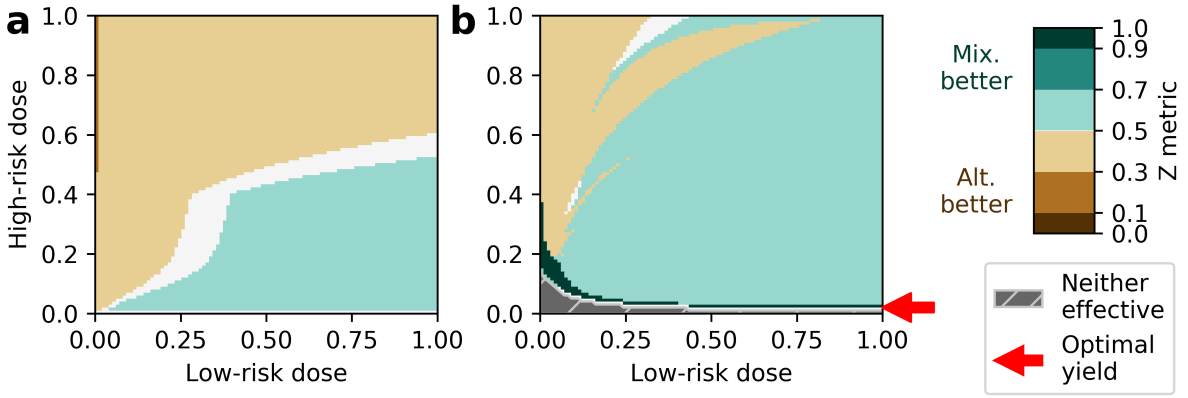

Figure 5.3: Relative performance of mixture and alternation of a high-risk and low-risk fungicide in the ElevenLeaf model. Tactics are compared by **a)** selection ratio and **b)** lifetime yield. The shaded-out area in **b)** shows where neither tactic manages a single season of effective control, and the red arrow shows the dose combination giving the overall optimal lifetime yield.

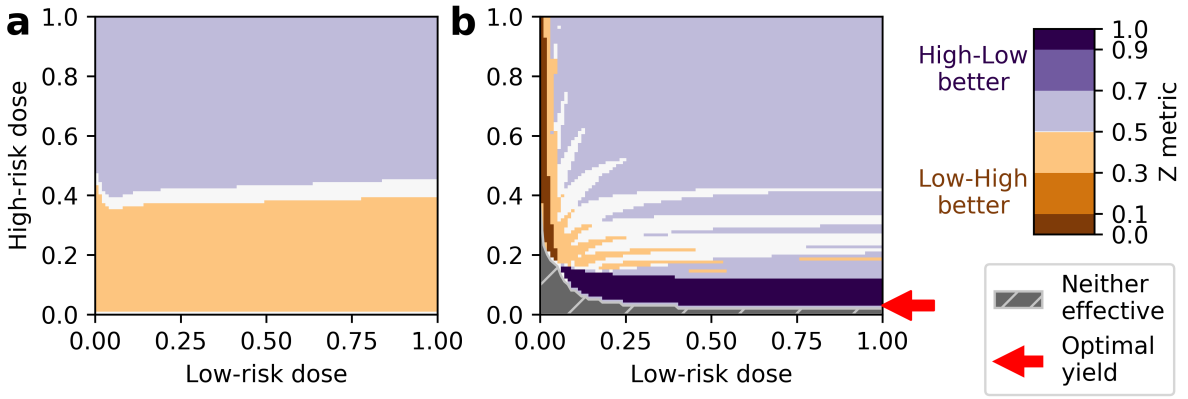

Figure 5.4: Relative performance of the two orders of alternation of a high-risk and low-risk fungicide in the ElevenLeaf model. Tactics are compared by **a)** selection ratio and **b)** lifetime yield. The shaded-out area in **b)** shows where neither tactic manages a single season of effective control, and the red arrow shows the dose combination giving the overall optimal lifetime yield.

risk is low then increasing the low-risk dose can increase the selection imposed significantly under the Alternation High-Low tactic (figure 5.7). This is due to the way that the resistance frequency that carries between seasons is calculated. The resistance frequency is calculated by using the resistance frequency across the infectious area on the top five leaf layers. This means that the resistance frequency in the next season is an average of the resistance frequencies of the top 5 leaves, weighted by the infection severity on each leaf. By applying an increased dose of low-risk as T1, the infection severity on the earlier emerging leaves is reduced relative to the later leaves. This increases the weighting of the later leaves toward the next season's resistance frequency. These later leaves have a higher resistance frequency as they intercept more of the T2 high-risk dose, and so the selection ratio is increased. Clearly although the final predictions may be similar, the mechanisms underlying

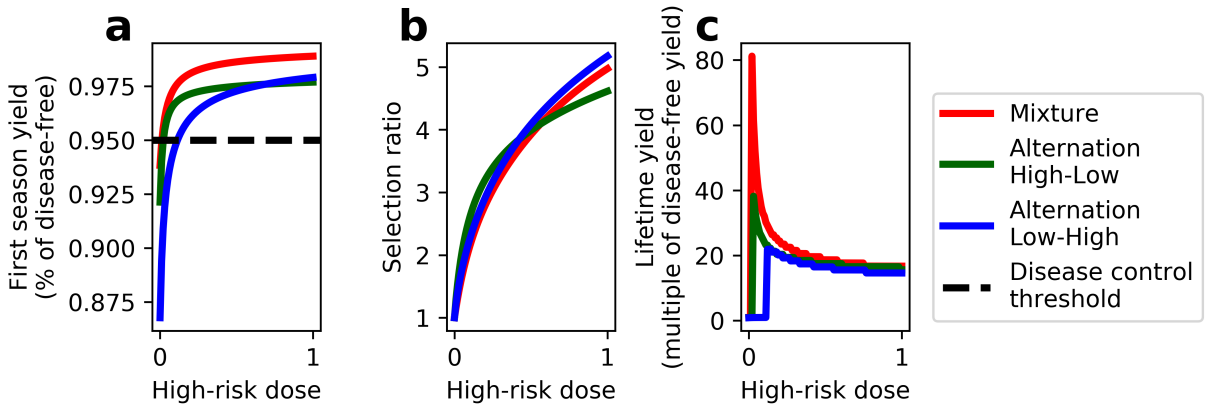

Figure 5.5: The performance of mixture and alternation in the ElevenLeaf model when the low-risk dose is maximal. Tactics are compared by **a)** first season yield, **b)** selection ratio and **c)** lifetime yield. The dashed line shows the critical yield below which disease control is considered to be ineffective.

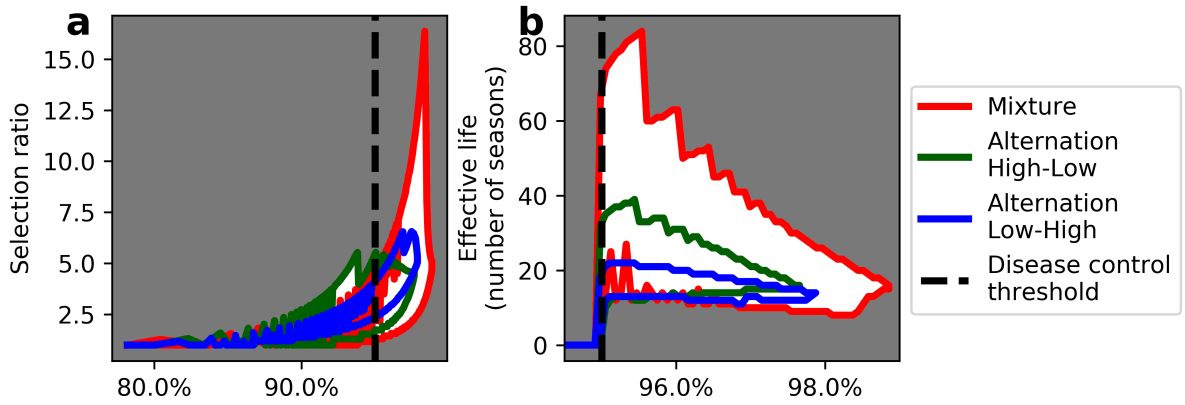

Figure 5.6: Performance of mixture and alternation in the ElevenLeaf model without the constraint of applying the same total dose. The performance of the tactics is shown in terms of **a)** selection ratio and **b)** lifetime yield. A range of values can be produced by each tactic for any given level of control; the polygons show the range that these values fall within for each tactic.

those results may be very different.

5.6 Discussion

5.6.1 The effect of primary inoculum

One of the model features included under the phenology umbrella term is the inclusion of the primary inoculum class (P). The inclusion of primary inoculum can lead to some counter-intuitive dynamics not seen in the simple model. In the simple model, the resistance frequency is increasing with time. However this is not necessarily the case in models with primary inoculum, as the primary inoculum can act as a reservoir of fungicide-sensitive pathogen. When a spray of high-risk fungicide is applied, the resistance frequency in the

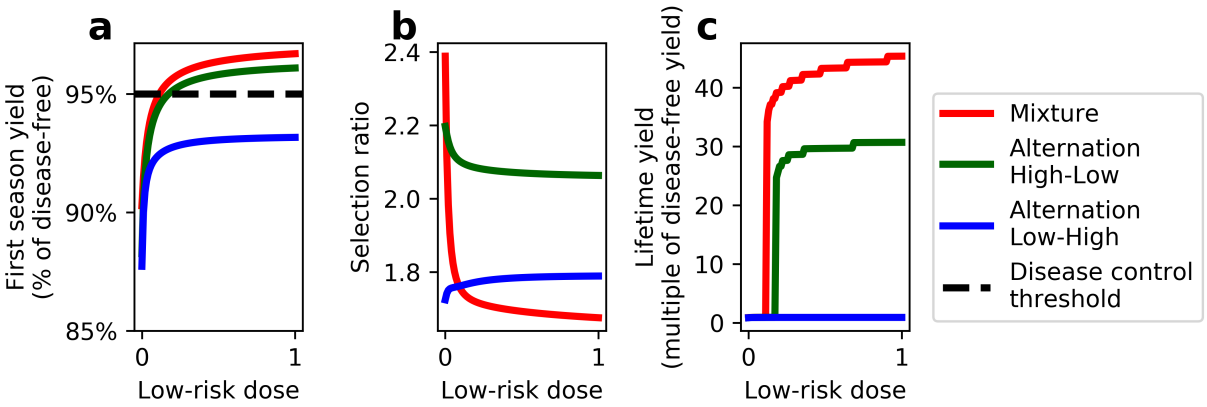

Figure 5.7: Performance of mixture and alternation in the ElevenLeaf model when the dose of high-risk is 0.05 and the low-risk dose is variable. Tactic performance is shown in terms of the **a)** yield in the first season **b)** selection ratio and **c)** lifetime yield. The dashed line in **a** shows the critical yield required for disease control to be considered acceptable. The lifetime yield for Alternation Low-High barely varies in **c** because there is no low-risk dose for which acceptable control is achieved, with the high-risk dose used.

secondary inoculum $\left(\frac{I_R}{I_R+I_S}\right)$ is increased relative to that of the primary $\left(\frac{P_R}{P_R+P_S}\right)$. As the high-risk fungicide decays away the per capita rates of primary infection for the fungicide-resistant and -sensitive strains become the same. The resistance frequency in the secondary inoculum then decreases to a weighted average between the resistance frequency in the primary and secondary inoculum. This average is weighted by the relative amounts of primary and secondary inoculum remaining.

Clearly the strength of this effect depends on the relative amounts of primary and secondary inoculum present when the high-risk fungicide is applied. This is one mechanism by which the timing of sprays can affect their impact on selection for resistance; earlier sprays occur when there is more primary inoculum remaining. Another way in which the inclusion of primary inoculum interacts with spray timing was discussed in section 2.4.2; we will return to this in the next chapter.

5.6.2 Deviations from the expected trade-off

The simple model presented in section 5.2 gives an analytical prediction of tactic performance for resistance management in terms of the trade-off between dose-splitting and suppression of growth rates by the mixing partner. By looking at the deviation of the predictions of the more complex models from the prediction of the simple models, we can determine what other factors apart from the expected trade-off may be affecting tactic performance. We shall refer to models by a string of characters specifying which features are included: **F** for fungicide decay, **H** for host-limited infection, **L** for latent period, and **P** for phenology.

As described previously, it is difficult to reconcile the doses applied in this simple model with those of a model including more a realistic treatment of fungicide decay. However this

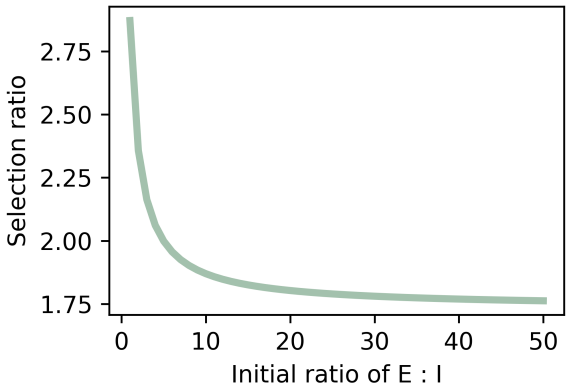

Figure 5.8: The effect of increasing the amount of primary inoculum at GS32 in the **F** model on the selection ratio of a single spray. The amount of primary inoculum is increased without reducing the amount of secondary so $E + I$ increases along the x-axis as well. A single spray of the high-risk fungicide at full dose is applied.

difficulty cannot explain the fact that as the dose of the low-risk gets very high in the **F** model, the range of high-risk doses for which alternation is favoured starts to increase. The trade-off in the simple model predicts that increased dose of low-risk always favours mixture. The difficulty in matching doses between models cannot explain this as any dose-transformation function must be increasing, and thus cannot change the sign of any derivative of curves in dose-space on transformation. As with the unexpected nature of the effect of changing the curvature parameter of the low-risk dose-response curve, this is due to the violation of the assumption that the fungicides from different sprays do not interact. There is in fact a degree of fungicide mixture under the alternation tactics. Despite the violation of this very basic assumption, the simple and **F** models still give very similar predictions.

Once fungicide decay is included, the next biggest effect on predictions comes from including latent infection. As well as allowing the consideration of the eradicant effect of the high-risk fungicide, the inclusion of latent infection affects the rate of resistance development in a non-trivial fashion. For example, increasing the amount of initial infection (E_R and E_S) in the **FL** model decreases the selection imposed by a single early spray of the high-risk fungicide (figure 5.8). This is particularly interesting as increasing the amount of initial infection leads to an initially increased per capita rate of growth for infectious tissue and so would be expected to increase the rate of selection according to the governing principles. This is because, similar to the effect of primary inoculum described before, the latent tissue can act as a reservoir of fungicide-sensitive pathogen.

One criticism of the above discussion might be that measuring the resistance frequency in the infectious tissue alone is the reason for the inability of the governing principles to predict some dynamics of the system. For example, if we considered the resistance frequency across all pathogen compartments (P, E, I) then the resistance frequency could not decrease within the season. However, because only infectious tissue (I) contributes to resistance in the next season this could still lead to the resistance frequency decreasing

over the seasonal boundaries.

Whether or not the infection rate is dependent on the available susceptible host tissue has little effect on the predictions. The simple model predicts that the rate of infection is unimportant, but this only holds if the rate of infection is the same at the different fungicide application times. The lack of effect of including host-limited infection suggests that the assumption of exponential growth may be reasonable, although the inclusion of host-limited infection does modulate the effect of timing on the selection imposed by a spray at a given time.

Although the effect of including or excluding host limitation of infection rates had little effect on the predictions, it is possible for this feature to lead to some very unintuitive effects. For example it is possible for an increase in the low-risk dose to lead to an increase in selection, rather than to have at worst no effect as would be expected. This is because the inclusion of host-limitation makes the growth rate of the pathogen strains at the time of a given spray dependent on the level of control imposed by previous sprays. If an earlier spray heavily suppressed the epidemic, then the per capita infection rate may be increased at a later spray due to an increased availability of host tissue. This would increases the magnitude of any selective pressure imposed by the second spray. An equivalent effect was observed in Carolan *et al.* (2017), where the use of fungicides at low levels could reduce the durability of host resistance. Ultimately these effects did not seem strong in the presented models, but they do make reasoning about the results of the models more complex, and might come more strongly into play in similar models of other pathosystems.

Overall the close match between the prediction of the full model and the simple model suggests that the trade-off between dose-splitting and suppression by the mixing partner contributes heavily toward determining the relative performance of mixture and alternation for resistance management. However the presence of other important effects is evident in that the alternation tactics perform differently as soon as fungicide decay is addded, and in every one of more complex models.

5.6.3 Conserved patterns between model yield predictions

The yield predictions of the models are more variable than those related to resistance management. Given the greater complexity inherent to yield prediction this is unsurprising. Most notable is the conservation of the same general pattern where both tactics fail at low doses of the high-risk, then mixture performs better as the dose is increased and finally alternation out-performs mixture. In all models this is driven by dose-splitting, with the same explanation as given for the full models in the previous chapter. The other key conserved pattern in yield prediction is that it is overall optimal to spray as much low-risk as possible and slightly more high-risk than is minimally required for initial disease control.

Chapter 5 Summary

- The broad patterns of tactic performance with regard to resistance management are well described by the governing principles.
- There are a number of mechanisms in the studied models that can lead to results unpredictable by the governing principles, although these only change the fine detail of the predictions.
- Comparing the predictions of nested simpler sub-models can be an effective tactic for understanding the predictions of more complex models.
- All models investigated predicted that the optimal strategy for lifetime yield is a mixture of as much low-risk fungicide as possible and just slightly more high-risk than required for initial control.

6

Understanding the effect of spray timing

> *It was the best of times, it was the blurst of times.*
>
> Mr Burns, *"Last Exit to Springfield", The Simpsons S4E17*, 1993

6.1 Introduction

6.1.1 The effect of spray timing in previous chapters

In the previous chapters comparing the performance of mixture and alternation, the main mechanisms driving the difference between the tactics were dose-splitting and suppression of the resistant strain by the mixing partner. Both of these factors are identical under each order of alternation, and yet the two alternation tactics perform differently. The only difference between the alternation tactics is the timing of application of the high-risk and low-risk fungicides. In this chapter we therefore investigate the effect of spray timing in greater detail.

6.1.2 The literature on spray timing

In general the guidance on spray timing is that treatments should be applied earlier rather than later, in order to manage fungicide resistance evolution (Brent & Hollomon, 2007a), although there appears to be no experimental evidence behind this recommendation (van den Berg *et al.*, 2013; Zlof & Sunley, 2011). The following mechanisms were initially proposed (Brent & Hollomon, 2007a).

1. When a systemic eradicant fungicide is mixed with a protectant fungicide, applying the mixture to existing infection means that the anti-resistance benefit of the mixing partner may be lost.

2. Applying the fungicide earlier is likely to suppress pathogen populations, reducing the population mutation rate and thus the likelihood of emergence of *de novo* resistance.

The first mechanism is applicable in both the emergence and spread phases of resistance development, whereas the second is only pertinent during emergence. Following our earlier

work, we will focus on the spread phase and thus might expect the first mechanism to have an effect.

The main modelling work on spray timing has been carried out by van den Berg and collaborators on the septoria leaf blotch and winter wheat pathosystem (van den Berg *et al.*, 2013, 2016). These authors suggested additional mechanisms for an effect of timing on resistance evolution.

1. Time-dependent changes in epidemic growth rate affect the strength of selection, as predicted by the governing principles.

2. Timing of sprays affects to what degree each leaf intercepts fungicide, thus changing the level of control and selection.

To investigate timing they used numerical simulation to firstly examine the effect of placing a single spray at a range of times and the performance of a T1 + T2 spraying tactic when the timings were shifted up to 9 days earlier or 18 days later than standard (van den Berg *et al.*, 2013). Secondly they simulated programmes involving all combinations of T0 – T3 sprays with both a high-risk and a low-risk fungicide (van den Berg *et al.*, 2016). The main conclusions of this work were the following.

- Standard T1 and T2 times can optimise fungicide effective life if appropriate doses are chosen.

- Earlier T1 and T2 sprays generally imposed less selection, although the exact pattern is complex.

- Optimal effective lives were generally produced by spraying at or close to the full emergence times of particular leaves. These timings also tend to optimise disease control.

- Some spray regimes impose less selection for a given level of disease control, although there is a close relation between the two.

- The inclusion of a T2 spray in a programme is key to maximising fungicide effective life.

- Programmes involving two sprays are superior to those involving three or four.

In van den Berg *et al.* (2013) the authors also notably conclude that for one particular fungicide dose tested, the timings that optimise disease control are also optimal for resistance management. However this does not agree with the presented data (figure 7 in their paper), which clearly show ranges of timings leading to the lowest losses to disease that are distinct to those with the greatest effective lives and lowest selection ratios. There are a wide range of times where both the effective life and control are reasonably high, but this is unsurprising as a non-zero effective life requires relatively high disease control and in fact around 90% of the timings investigated lead to an effective life only one season less than the maximum.

6.1.3 The aims of this chapter

In this chapter we will first briefly demonstrate how the governing principles can be used to explain the effect of timing seen in the model used in the previous chapters.

Next we will examine in more detail the effect of timing in a more complex model. The model used in the van den Berg *et al.* papers tracks each of the upper leaf layers explicitly, as the timing of fungicide sprays relative to leaf emergence was expected to be an important factor in controlling the impact of spray timing. We adopt a later version of this model, with more explicit consideration of the lower leaf layers and a more accurate treatment of the effect of fungicide on them (see section 2.4.3). We will investigate how timing in programmes involving one or two sprays of a high-risk fungicide affects performance. We will investigate a broader range of time combinations in the two spray regimes than van den Berg *et al.* (2016), and will use timing as a motivating case for testing the predictive power of the governing principles.

Finally we will use the understanding gained from looking at one and two sprays to investigate the interaction between spray timing and the question of mixture or alternation.

6.2 FiveLeaf model

6.2.1 Explaining the effect of timing with the governing principles

Before investigating the more complex model, we first briefly examine the effect of timing in the FiveLeaf model to explain the differences between the two orders of alternation that were observed in previous chapters.

There is a strong effect of spray timing on the selection and control imposed by a given dose of fungicide (figures 6.1 and 6.2). We considered spray times ranging from the emergence of leaf 3 to 6.64 ($\approx \log 100/\log 2$) half-lives of the fungicide before the end of the season. That particular final time was chosen as it allows the fungicide to have decayed to at least 1% of its initial concentration by the end of the season. This reduces the edge effect of the removal of fungicide at the end of the season.

The time trend for the selection imposed by a spray is much simpler, and opposite, to that seen in van den Berg *et al.* (2013). Earlier sprays impose greater selection than later. This can be explained by the governing principles, as the per capita growth rate of the pathogen decreases with time in this model. Note that there is no unambiguous reason to select any particular compartment of combination of compartments to consider for use with the governing principles in a multi-compartment model, but all but one possible combination of compartments for growth rate calculation show a very similar trend with time (figure 6.3). We therefore opt for considering the growth rate of the infectious tissue alone, as this is the only infected compartment that contributes to resistance in the next season. There is a close relationship between the per capita growth rate of the pathogen at a particular time and the

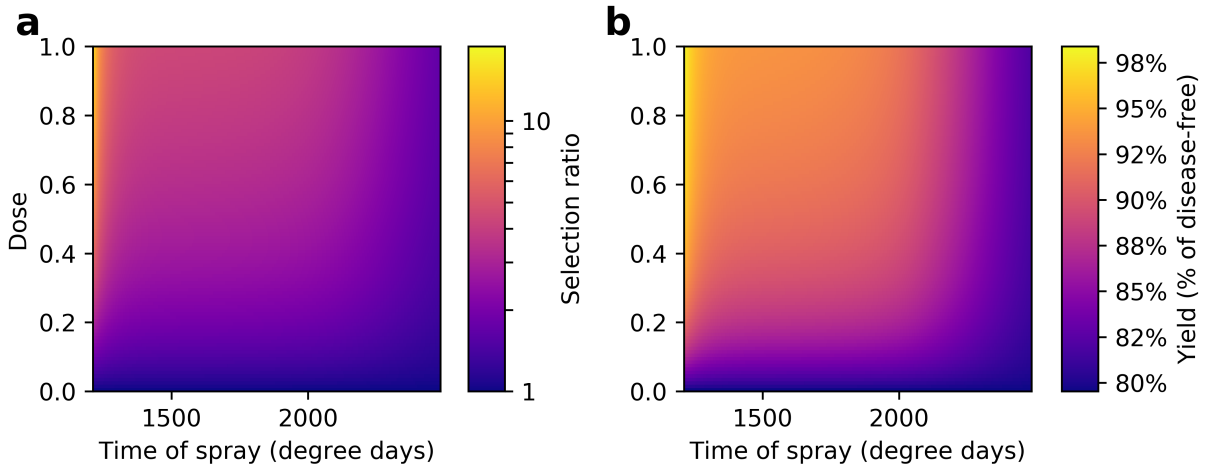

Figure 6.1: The effect of changing dose and timing of a single spray of pyraclostrobin in the FiveLeaf model. **a)** The selection ratio and **b)** the yield from applying a single spray at a given time and dose, with the range of times restricted to remove the edge effect of the end of the season.

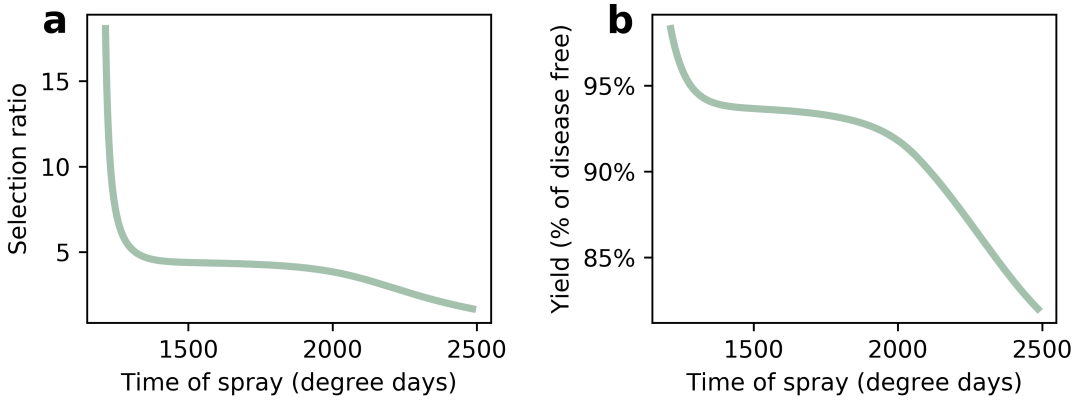

Figure 6.2: **a)** The selection ratio imposed and **b)** the yield produced by a single full dose spray of pyraclostrobin at a range of times in the FiveLeaf model, with the range of times restricted to remove the edge effect of the end of the season.

selection pressure imposed by a spray at that time as predicted by the governing principles (figure 6.4).

6.2.2 The relationship between selection and control

There is a strong mechanistic connection between the disease control imposed by a spray and the selection pressure it imposes. It is therefore unsurprising that these outputs show similar responses to the dose and time of a spray (figure 6.1). However this begs the question of whether the relationship is strictly monotonic, or whether it is possible to reduce the selection imposed by a spray without sacrificing control by modulating either the dose or the application time. One mechanism for decoupling selection and control is the use of a low-risk fungicide as a mixing or alternation partner, but here we focus only on the use of a

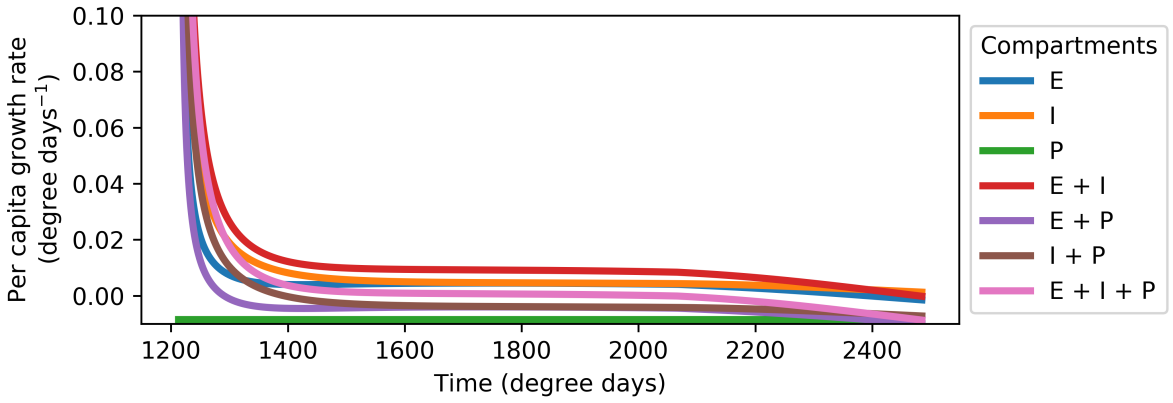

Figure 6.3: The per capita growth rates of all combinations of pathogen compartments over time in the FiveLeaf model. In each case the per capita growth rate is defined as

$$\frac{\sum_{i\in\Omega} \frac{\mathrm{d}}{\mathrm{d}t} X_i}{\sum_{i\in\Omega} X_i},$$

where X_i is a compartment and Ω is the set of compartments considered.

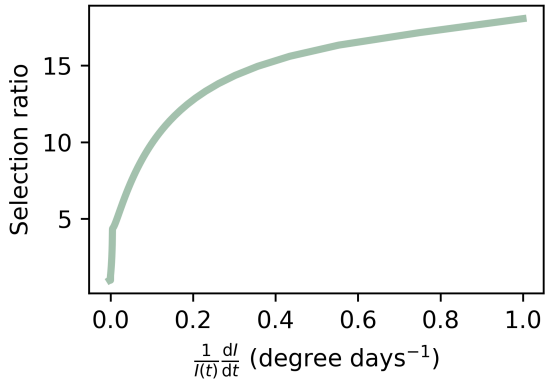

Figure 6.4: The selection ratio imposed by a single full dose spray of pyraclostrobin in the FiveLeaf model compared to the per capita growth rate of infectious tissue at the time of that spray. Sprays were applied over the range of times shown in figure 6.2.

single high-risk fungicide.

We will describe the relationship between selection and control by a metric we term the spray efficiency,

$$\text{Spray efficiency} = \frac{\text{Yield under tactic} - \text{Yield under no control}}{\text{Selection ratio under tactic}}. \tag{6.1}$$

This is similar to the graphical method of van den Berg *et al.* (2016), where spray regimes were compared by fitting a straight line through the relationship between the logarithm of the selection ratio and loss of yield to disease for a range of spraying regimes (figure 6.5). Regimes below that line were considered to good candidates for long effective lives. Note that the spray efficiency is related but different to the concept of dose efficiency in van den

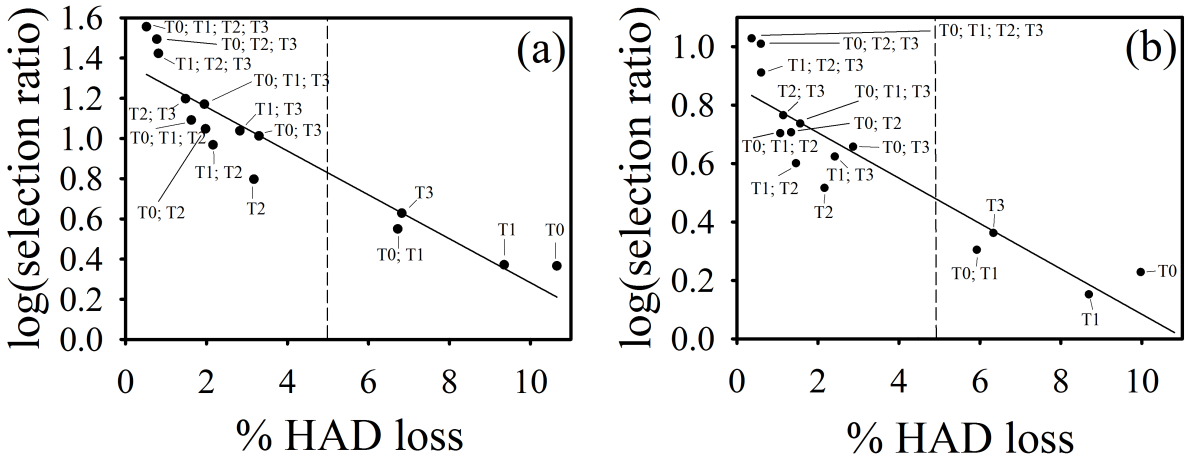

Figure 6.5: The relationship between the selection ratio and loss of healthy area duration (closely related to yield) in the tactics investigated in van den Berg *et al.* (2016). A straight line of best fit is put through the data points, identifying points below the line as imposing less selection for the level of disease control than the average. Figure reproduced from van den Berg *et al.* (2016) with permission of authors.

Berg *et al.* (2013), which is the reduction in AUDPC per unit dose.

Modulating the time of dose of a single spray leads to variation in spray efficiency (figure 6.6). Notably for any given time of application there is an optimum dose for maximising spray efficiency, and for any given dose there is an optimum time. The most efficient dose varies with application time and vice versa, reinforcing that spray timings and doses cannot be optimised independently (figure 6.7).

6.2.3 Implications

The above shows that even without the increased complexity introduced in the next sections, timing can have a large effect on the impact of a spray with regards to selection and control. In addition it shows that greater disease control does not necessarily mean a concomitant increase in selection. When attempting to reason about the effect of a particular spray timing and dose, the governing principles therefore provide more predictive power than thinking in terms of control alone. The governing principles explain why when comparing the two orders of alternation in the previous chapters, the alternation applying the high-risk later in the season was generally better for resistance management.

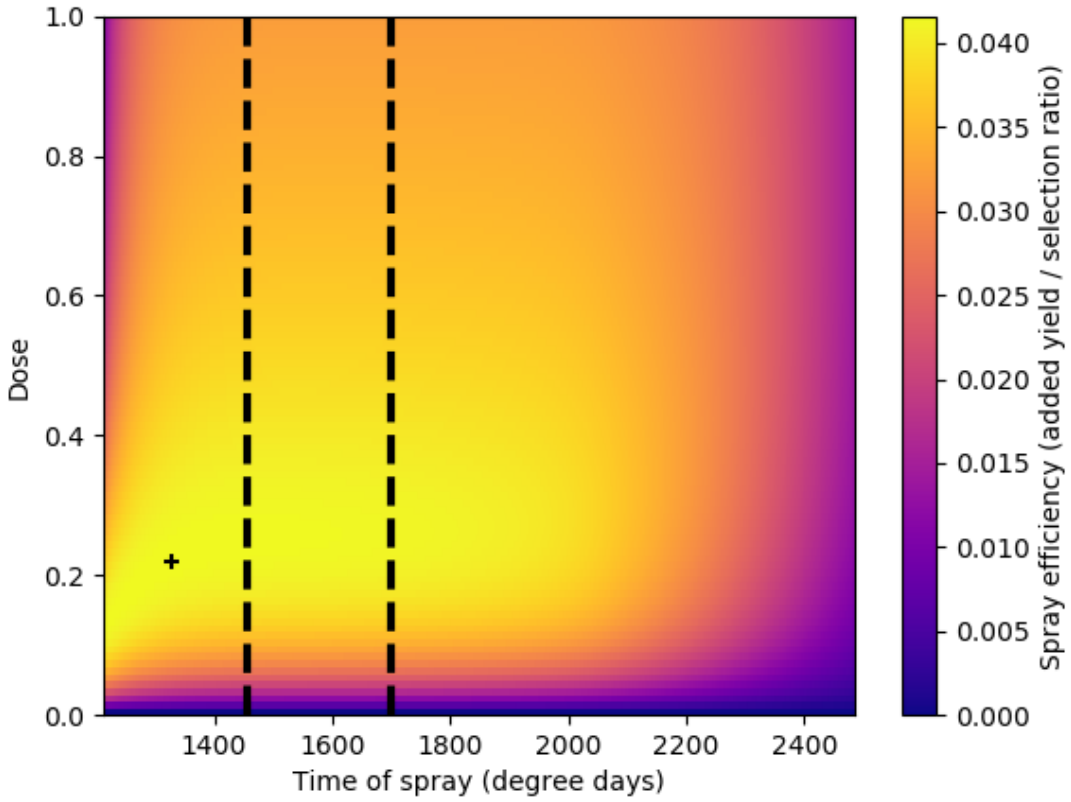

Figure 6.6: The spray efficiency for a single spray of fungicide in the FiveLeaf model at a range of application times and doses. The dashed lines showed the times at which a T1 (GS32) and T2 (GS39) spray would be applied. The cross shows the position of the maximum spray efficiency.

6.3 ElevenLeaf model

6.3.1 Timing of a single spray

In the FiveLeaf model, the governing principles are very effective at predicting which fungicide application times impose a greater selection pressure than others (figure 6.4). However the ElevenLeaf model demonstrates some behaviours not possible in the FiveLeaf model, for example that the use of a low-risk fungicide can increase the strength of selection for resistance to the high-risk as seen at end of the the previous chapter. We are therefore interested in the extent to which the governing principles can be used to understand the predictions of this more complex model. To do so we carry out the same analysis as with the FiveLeaf model, placing a single spray of a high-risk fungicide at different times in the season and relating the selection ratio produced to the pathogen growth rate at the time of application.

As in the FiveLeaf model, there is a clear effect of timing on the selection pressure imposed by a spray and the increase in yield that results (figure 6.8). There is a more

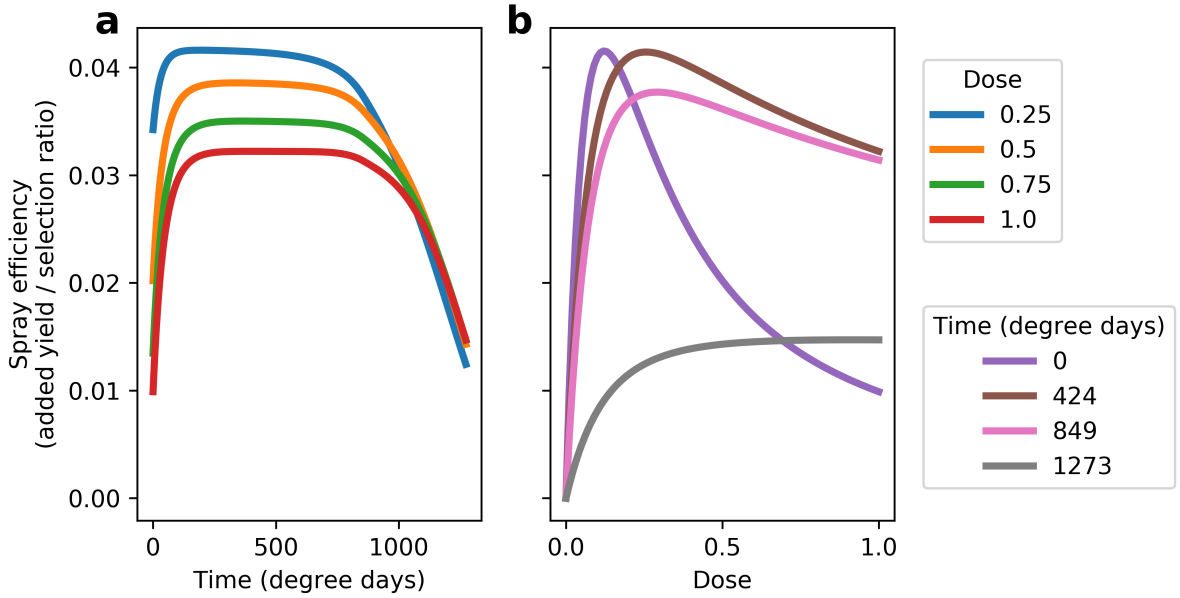

Figure 6.7: The spray efficiency for a single fungicide application in the FiveLeaf model when **a)** dose or **b)** spray timing is fixed. The optimal dose for spray efficiency depends on the time of application and vice versa.

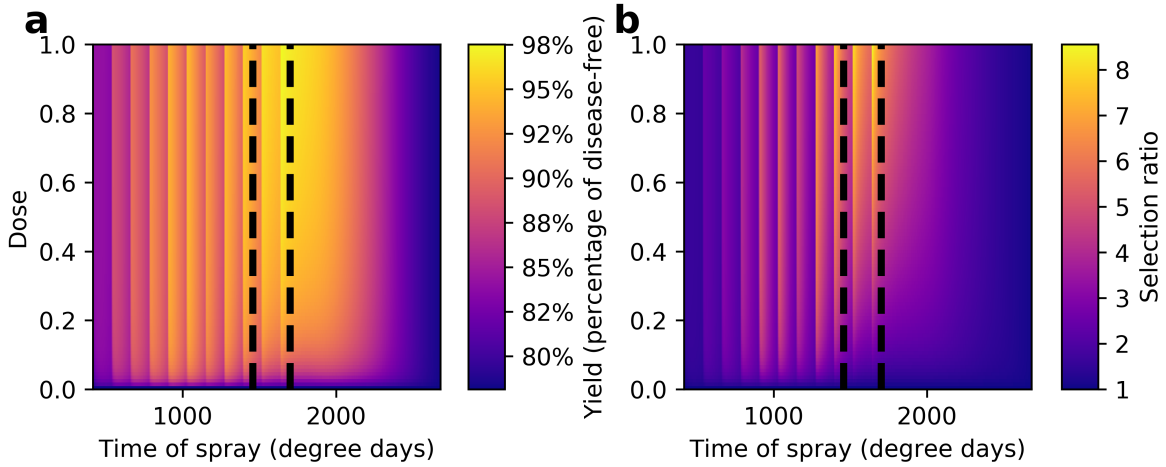

Figure 6.8: The effect of changing dose and timing of a single spray of high-risk fungicide in the ElevenLeaf model. **a)** The yield and **b)** the selection ratio from applying a single spray at a given time and dose. The dashed lines show the standard timings for a T1 and T2 spray.

complex pattern of change of spray efficiency with timing and dose than the simpler model (figure 6.9). The pattern for the selection imposed and yield permitted by a spray over time is also much more complex than in the FiveLeaf model, with clear peaks in both metrics coinciding with the emergence of each leaf layer. This is in part explained by the fact that the earlier a spray is applied in a leaf's lifecycle the more protection it imparts to that leaf and also that sprays applied before a leaf emerges cannot protect it. There are other factors at work too, for example the effect of timing on the effect of the fungicide which is demonstrated later in this chapter.

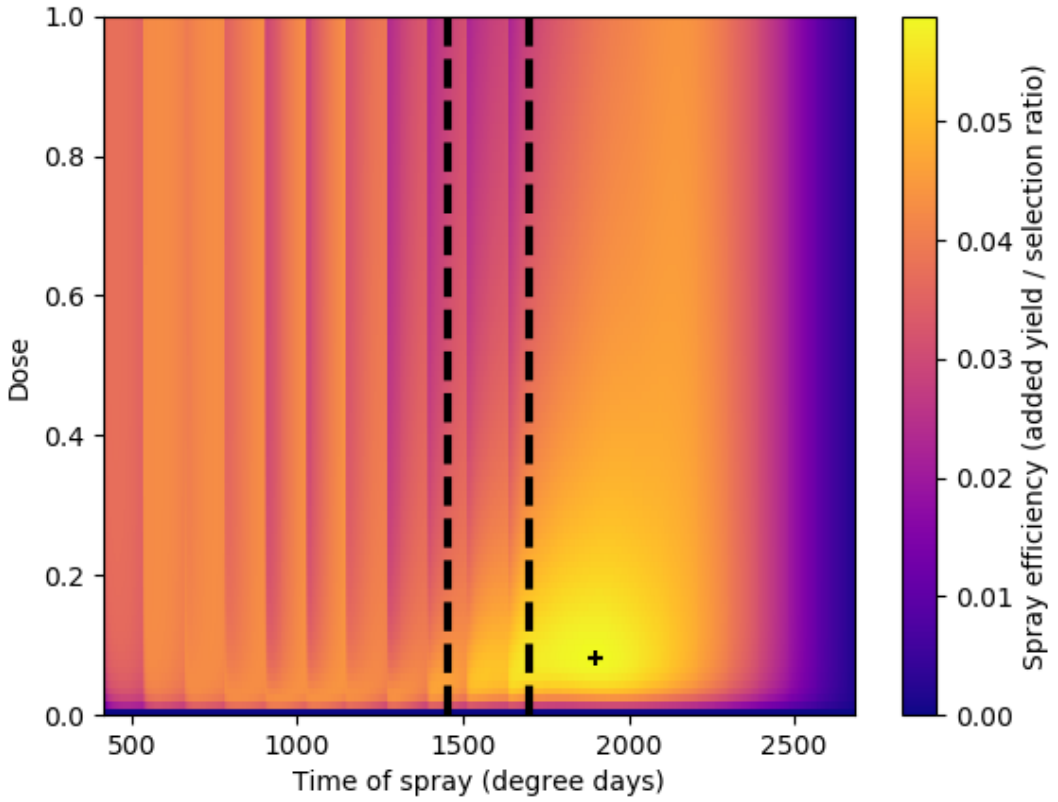

Figure 6.9: The spray efficiency of applying the high-risk fungicide at a range of times and doses in the ElevenLeaf model. The dashed lines show the standard T1 and T2 timings, and the cross shows the time and dose leading to the greatest spray efficiency.

Applying the governing principles to the ElevenLeaf model is intrinsically difficult as there are a large number of infected compartments to consider and the dynamics of these compartments are much more complicated. We shall investigate three reasonable possibilities for which rates to use with the governing principles.

- The per capita rate of change across all infectious tissue ($\dot{I}_A$),

$$N(t) = \sum_{i=1}^{11} I_i(t) \tag{6.2}$$

$$\dot{I}_A = \frac{1}{N(t)} \frac{\mathrm{d}N(t)}{\mathrm{d}t} . \tag{6.3}$$

- The per capita rate of change of the infectious tissue on the most recently emerged leaf ($\dot{I}_U$),

$$U(t) = I_i(t) \text{ where } i = \sup\{i | i \in \{1..11\} \text{ and } A_i(t) > 0\} \tag{6.4}$$

$$\dot{I}_U = \frac{1}{U(t)} \frac{\mathrm{d}U(t)}{\mathrm{d}t} . \tag{6.5}$$

- The average per capita rate of change of the infectious tissue on each leaf weighted by the amount of fungicide each leaf intercepts ($\dot{I_W}$).

$$F(i,t) = (1 - \mathrm{e}^{-\tau A_i}) \left(\prod_{j=1}^{i-1} \mathrm{e}^{-\tau A_i} \right) \tag{6.6}$$

$$\dot{I_W} = \frac{\sum_{i=1}^{11} \left(F(i,t) \frac{1}{I_i(t)} \frac{\mathrm{d}I_i(t)}{\mathrm{d}t} \right)}{\sum_{i=1}^{11} F(i,t)} . \tag{6.7}$$

Note that we amalgamate the resistant and sensitive sub-compartments for the calculation of these rates.

In the simpler FiveLeaf model the per capita growth rates of all infected compartments (apart from P) decreased with time, but in the ElevenLeaf model these rates show more complicated dynamics (figure 6.10). The growth rate across all layers is decreasing with time. It is infinite at the beginning of the season when there is no infectious tissue, and then decreases as infection spreads. In the same way, the growth rate on the most-recently emerged leaf and the rate weighted by fungicide interception peaks with each newly emerged leaf.

Ultimately the effects of other mechanisms controlling the selection imposed by a spray reduce the correlation between the growth rates and selection (figure 6.11). The per capita rates measured on the leading leaf and the weighted average do show a relationship between the growth rate at the time of a spray and the selection imposed, but this relationship varies between leaves. The growth rate on any particular leaf is closely correlated with the age of that leaf. This makes it difficult to disentangle the effect of pathogen growth rate and other factors associated with leaf age, such as proportionally more of the dose being lost on leaf removal as the spray timing gets later in the leaf's lifetime. Ultimately the variation in the relationship between the growth rates and the selection imposed for different leaves shows that the governing principles alone are not sufficient to predict the impact of a spray in this case. Note that the growth rates would still have the same effect as in the simpler model if all else were equal between spray timings. Factors that affect the growth rate at all times equally, such as a greater base infectiousness of lesions, still therefore follow the predictions of the governing principles.

There are a number of complex effects which connect leaf structure at the time of application and the impact of a given spray. We shall describe one to demonstrate how dose and time can interact. Not all of the fungicide applied by a given spray is actually intercepted by the leaves; the total amount intercepted and the distribution over the different leaf layers depends on the time of application (see equation 2.29 in section 2.4.3). Therefore the actual active dose is diluted by a time-dependent factor. The concave relationship between fungicide and effect means that a fixed proportional reduction in dose can have varying

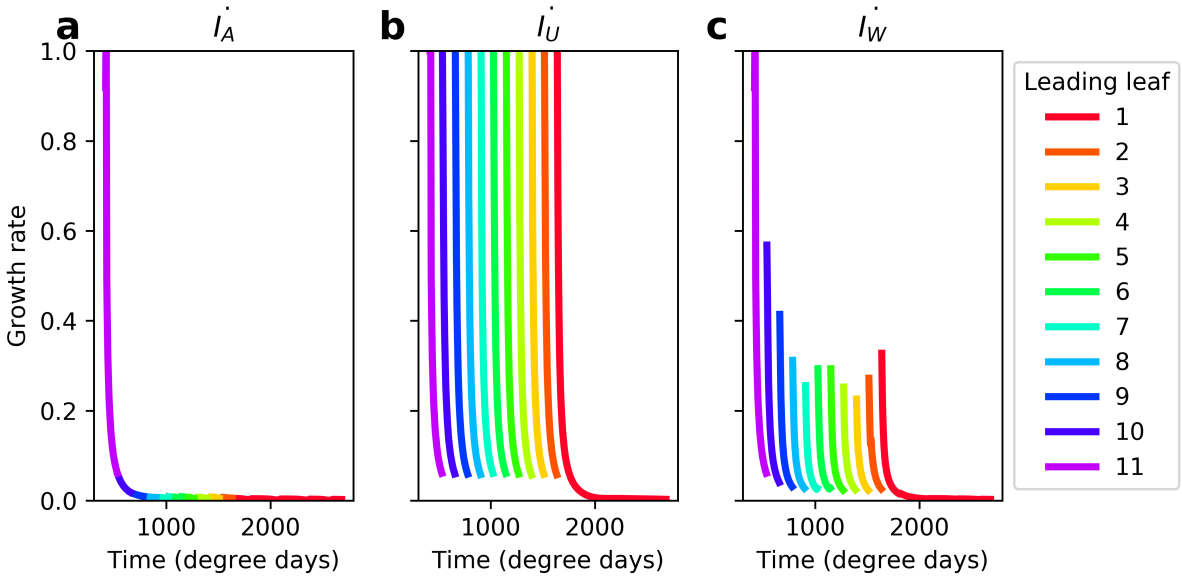

Figure 6.10: The change in the per capita growth rates for **a)** infectious compartments across all leaf layers, **b)** the infectious compartment on the most recently emerged leaf layer and **c)** infectious compartments across all leaf layers weighted by the amount of fungicide each layer intercepts. The data is split into subsets (differentiated by colour) according to the emergence of each new leaf.

degrees of impact on the effect of the fungicide, depending on the dose applied. The fact that different amounts of fungicide are intercepted by each leaf layer at the T1 and T2 spray, can create the case as above where depending on the dose applied either timing can can lead to a lesser selective pressure. Furthermore, concave dose-response curves mean that distributing fungicide over a greater number of leaf layers has an equivalent effect to splitting doses over multiple sprays; the same total concentration divided over a greater number of layers imposes more effect on the pathogen. The relationship between the total effect imparted on the pathogen and the time of spray is complex and shows a pattern distinct to the relationship between the total concentration of fungicide and spray time (figure 6.12).

6.3.2 Extension to two sprays

We have investigated in detail the effect of changing the timing of a single spray of fungicide. We now consider two sprays of fungicide, to investigate the extents to which the patterns observed in the single spray case apply in more complex situations. In addition, the use of two sprays represents a more realistic situation and provides sufficient control to allow us to investigate the impact of timing on longer term aspects such as lifetime yield.

With two sprays there are four independent variables to investigate (two times and two doses) and based on the above the effect of each will depend on the choice of the others. This makes visualisation of the data difficult and so we reduce dimensionality by looking at optimal situations. We do this by either varying the timings of each spray and choosing the

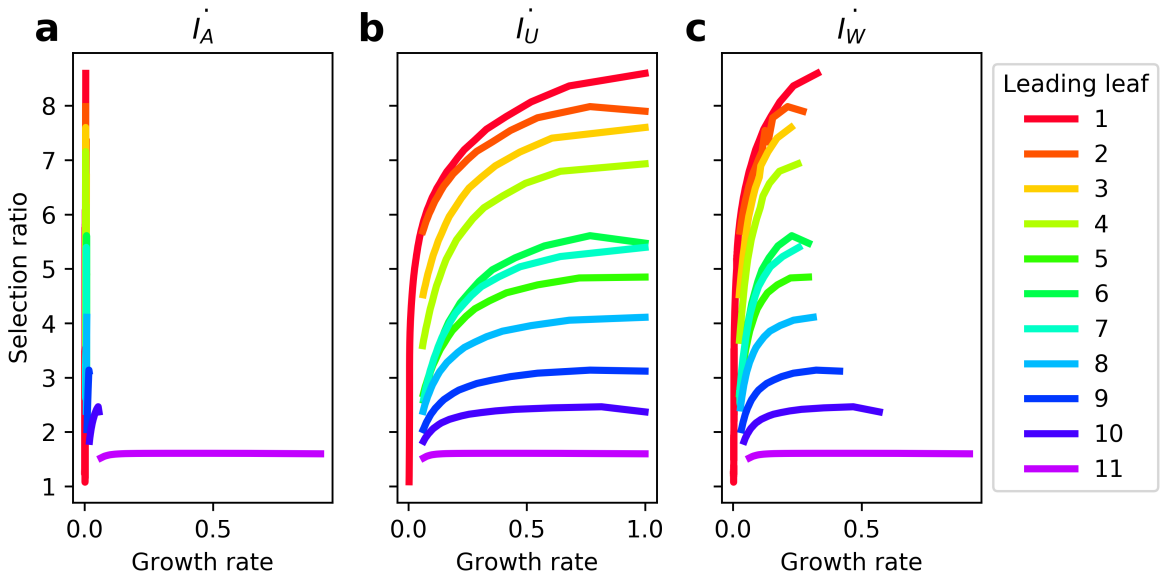

Figure 6.11: Relationship between the selection ratio and the per capita growth rates for **a)** infectious compartments across all leaf layers, **b)** the infectious compartment on the most recently emerged leaf layer and **c)** infectious compartments across all leaf layers weighted by the amount of fungicide each layer intercepts. The relationship is shown for the case of a full dose of fungicide being applied. The data is split into subsets (differentiated by colour) according to the emergence of each new leaf. The value of the growth rate is varied by altering the spray timing over the same range as in figure 6.10.

doses that produce the best possible value for any given metric of comparison (figure 6.13), or varying the doses and choosing the optimal timings (figure 6.14). Note that the selection ratio is not shown as it can be trivially minimised with any choice of times or doses. There is a clear critical period, between around 1650 to 1950 degree-days into the season, in which a spray must be applied to achieve the highest effective life. The flag leaf emerges 1635 degree-days into the season, and so this critical period highlights the important of protecting the flag leaf for yield generation. From figure 6.9 it is also clear that this time range provides a high level of control for the amount of selection imposed.

The tactic leading to the overall optimal lifetime yield was to apply a dose of 0.08 at 1709 and of 0.13 at 1959 degree-days into the season. The exact values are not too important as they are in part decided by the resolution at which the simulations were run. As seen in the previous chapters the optimal dose of fungicide is relatively low. These spray timings and doses both fall into the area of high spray efficiency identified before (most yellow part of figure 6.9)). The effect of multiple sprays are not independent, which is why it is not optimal to place two sprays at the same optimal time as for a single spray. Furthermore spray efficiency can identify a dose-time combination as being a candidate for a high lifetime yield, but it is not necessarily optimal for lifetime yield to optimise spray efficiency.

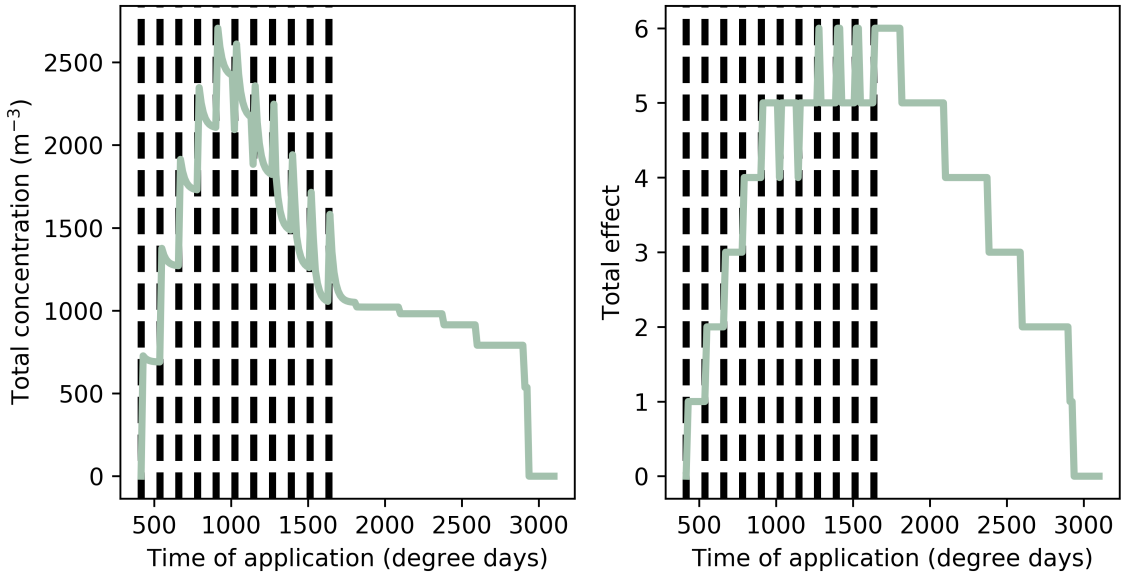

Figure 6.12: The effect of spray timing on the initial **a)** total concentration and **b)** total effect across all leaf layers. The total concentration is the sum of the concentration on each layer, and the total effect is the sum of the effect imposed on each layer. The dashed lines show the times of emergence of each leaf layer.

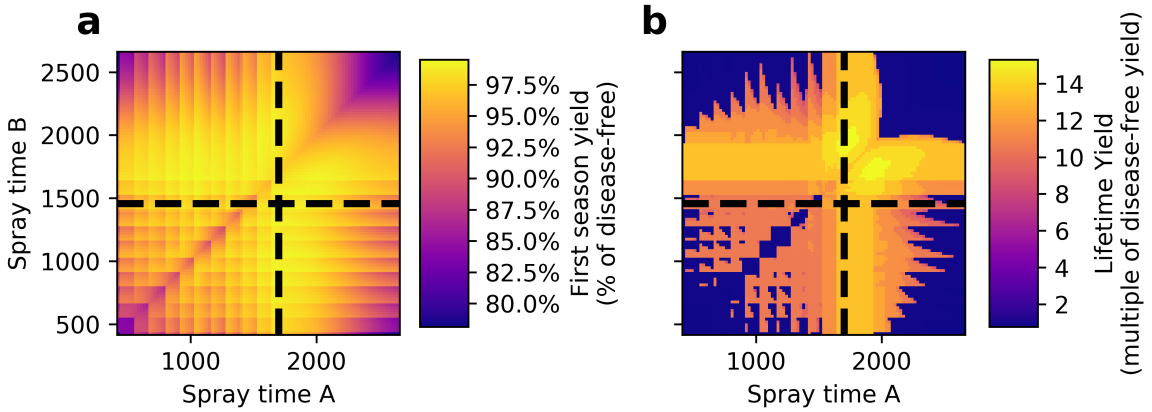

Figure 6.13: The largest **a)** first season yield and **b)** lifetime yield that can be achieved for two sprays of fungicide in the ElevenLeaf model at a range of times. The dashed lines show the default timings for T1 and T2.

6.4 Mixture and alternation with variable timings

We have seen in this chapter that the timing of sprays can have a large effect on their performance. We have also previously shown in chapter 5 that the ElevenLeaf model shows the same general predictions for the relative performance of mixture and alternation as the FiveLeaf model, although the details differ. In this section we investigate how spray timing interacts with the question of which of mixture and alternation is better. There are two main motivating situations for this investigation. The first is planned proactive changes in timing made in order to get a better result. The second is an unplanned reactive change in timing,

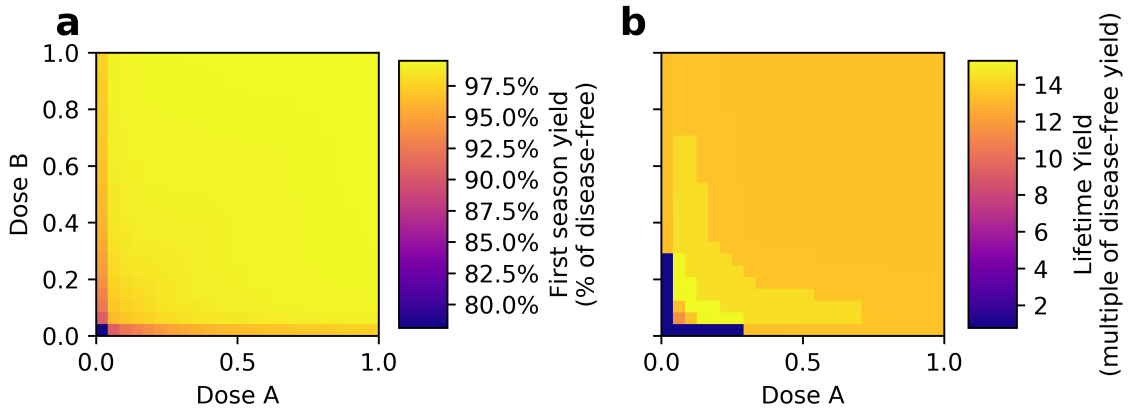

Figure 6.14: The largest **a)** first season yield and **b)** lifetime yield that can be achieved for two sprays of fungicide in the ElevenLeaf model at a range of doses.

for example due to weather unsuitable for spraying. In either case we are interested in whether mixture or alternation performs better, but the way in which the comparison is posed will vary.

Simulations were run for two sprays of fungicide at a range of doses and times between the emergence of the first leaf and a time-point sufficiently far from the end of the season to allow the fungicide to have decayed to 1% of its initial concentration. For each pair of timings, mixture and the two alternations of a low-risk and high-risk fungicide were simulated. Firstly we examine the case of a proactive change in spray timing, in which case we seek the overall optimal combination of timing and dose. For the range of tactics investigated this is to apply a mixture of a full dose of low-risk and a very low dose (0.04) of high-risk with the sprays timed at 1664 and 2186 degree-days. This tactic leads to an initial level of control just slightly higher than the critical value of 95%, and so represents the same optimal tactic as seen in the previous chapters. That is, to apply as much of the low-risk fungicide as possible and just slightly more high-risk than initially required. We note that the optimal timings are different to when applying two sprays of the high-risk fungicide, which again highlights that timing cannot be optimised without taking into account the doses and particular fungicides being used. Although the timings are different to those which are optimal for two sprays of the high-risk they yet again fall in the later part of the season, both being later than a standard T2 timing.

Secondly we look at the situation of a reactive change in timing. Whereas before we were free to choose the optimal timings, we now assume that timings are fixed and look at the optimal tactic for those timings. In almost all cases the optimal tactic for any given pair of timings is to apply a mixture rather than alternation (figure 6.15). The optimal tactic always involved spraying as much low-risk as possible (figure 6.16). When the sprays were timed such that the initial yield could exceed 95% the optimal high-risk dose was just slightly higher than required for that yield, and when reaching the threshold was not possible the optimal tactic was to apply as much high-risk as possible, maximising the yield over the

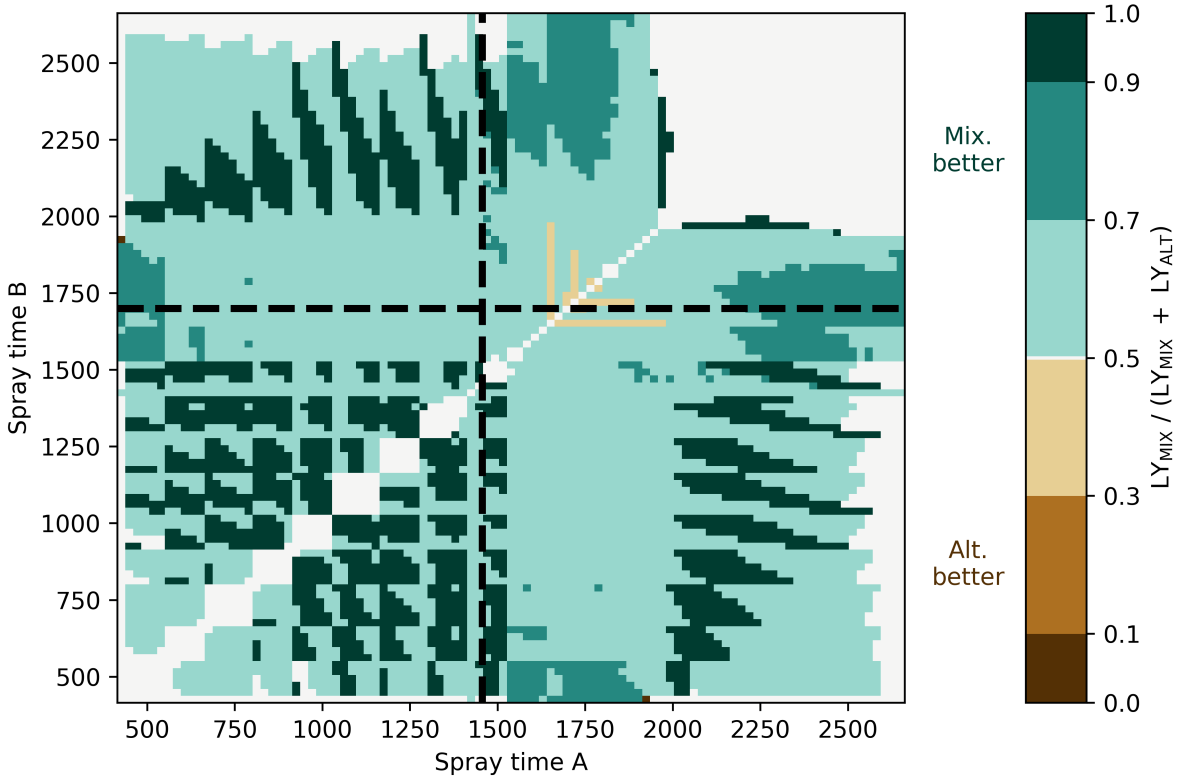

Figure 6.15: The relative performance of the best alternation and mixture tactics for a given pair of spray timings. The dashed lines show the standard T1 and T2 timings. Note that the graph is perfectly symmetrical along the diagonal from bottom left to top right. Switching the sprays under mixture has no effect as they are identical. Switching the sprays under alternation just switches which alternation tactic the regime represents, and both alternations are considered at every pair of spray times.

single season of use.

The close connection between timing and dose when optimising a fungicide application tactic is again shown by looking at the performance of mixture and alternation for fixed doses (figure 6.17). The flexibility in spray timing leads to a very different pattern for the relative performance of mixture and alternation in dose-space compared to fixed spray timing (*cf.* section 5.5). We note that it is an unrealistic situation to have fixed dose but variable timing, but it serves as a good point of illustration.

It was mentioned in the introduction that one possible explanation for an effect of timing on the selection pressure imposed by a spray was that later sprays might mean that the protectant action of a mixing partner is lost and thus no longer suppress the resistant strain. This mechanism would lead us to expect to see alternation performing relatively better later in the season, as mixture benefits much more from suppression by the partner fungicide. However this trend was not observed (figure 6.18).

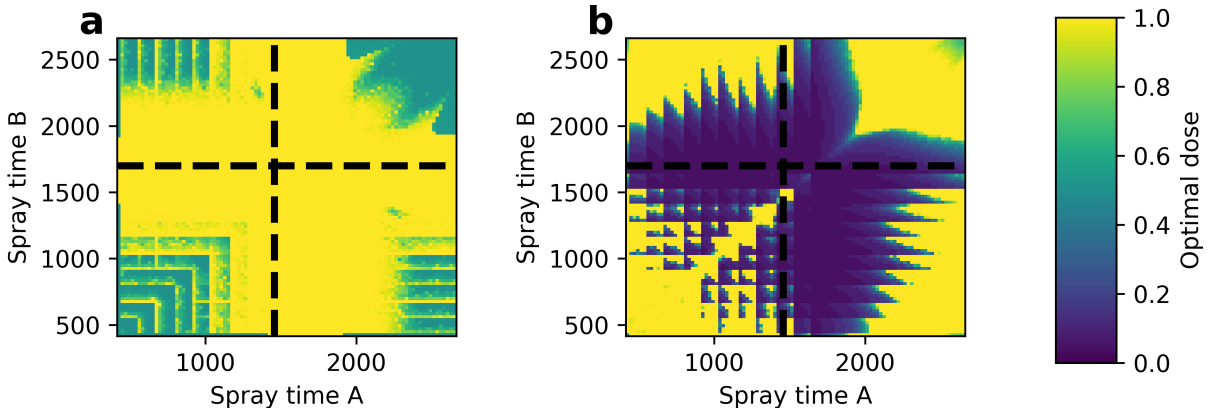

Figure 6.16: The dose of the **a)** low-risk and **b)** high-risk applied in the optimal tactic (mixture or alternation) for a range of spray times. The areas where the optimal low-risk dose is less than one are where multiple doses lead to the same optimal outcome, and so the average dose is less than one. The areas where the optimal high-risk dose is very high are where the threshold yield of 95% cannot be reached, and so control is maximised without regard to selection.

6.5 Discussion

6.5.1 The usefulness of the governing principles for high resolution predictions

In the previous chapters we used the governing principles to explain the relative impact of different fungicide application regimes on resistance development. In this chapter we put the governing principles through a quantitative test, examining how well the growth rate of pathogen at a particular time correlates with the selection pressure imposed by a spray at that time. In the simpler FiveLeaf model of septoria, the per capita growth rate was a very good predictor of strength of selection. In the more complex ElevenLeaf model, the complicated host tissue dynamics introduce a number of confounding factors that weaken the predictive power of a simple application of the governing principles considerably.

The ineffectiveness of the governing principles in the ElevenLeaf model is partly due to the exact technique used. The governing principles were conceived with exponential growth of a single infectious compartment in mind, and the extension to more complex multi-compartment models is non-trivial. A number of different possibilities exist but each has its own flaws. For example if one considers the rate across all infected compartments, then the fact that in both the FiveLeaf and ElevenLeaf model only the infectious compartments contribute to the resistant population in the next season can lead to unexpected results. Considering only the compartments that contribute to resistance in the next season may therefore seem appropriate, but this can mis-identify positive selection that reduces in intensity over time as negative selection (*e.g.* see examples in chapter 5).

Aside from issues of choosing which compartments to consider, the predictive power is

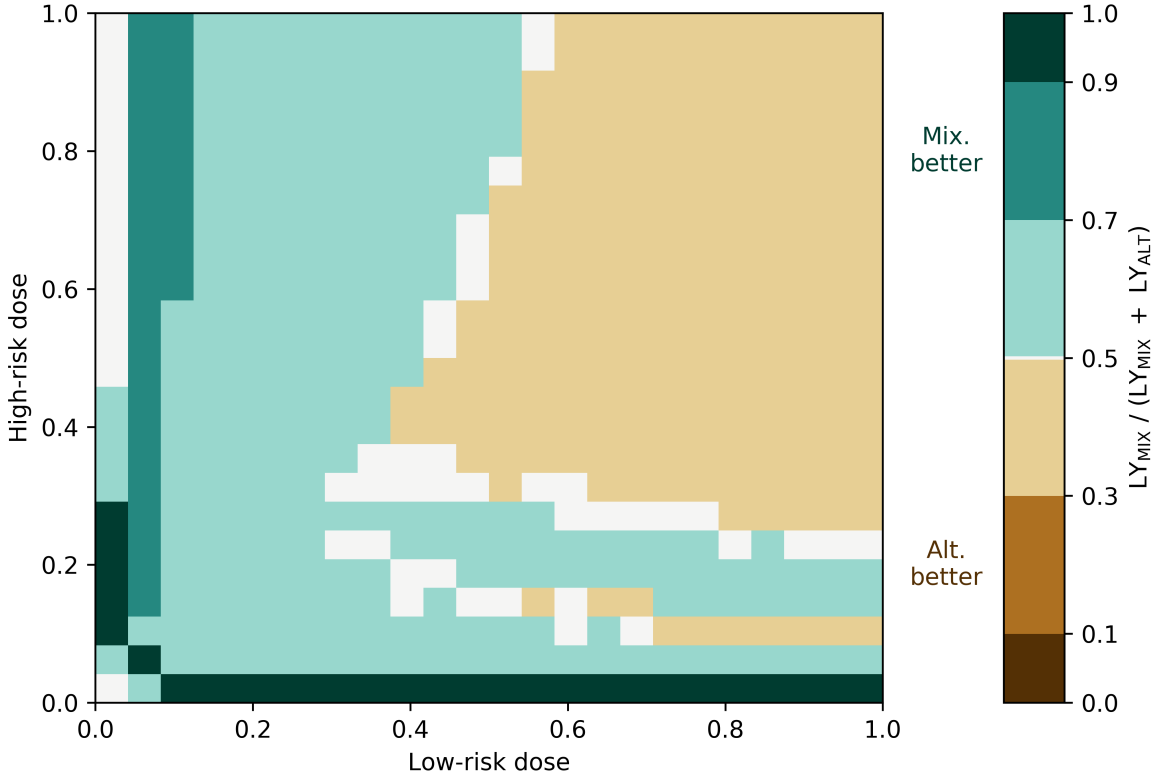

Figure 6.17: The relative performance of mixture and alternation in the ElevenLeaf model when the timing of the two sprays is flexible. For each pair of doses the comparison is made between the best lifetime yield possible under each tactic. The optimal spray times for each tactic are not necessarily, and are in general not, the same.

weakened by necessary simplifying assumptions. Implicit to our correlation of the per capita growth rates and selection ratios in this chapter is the assumption that the per capita growth rate when fungicide is applied is a good indicator of the per capita growth rate for the entire time fungicide is present. The accuracy of the predictions could certainly be improved by measuring the per capita growth rate over the entire time the fungicide is present, but this of course somewhat defeats the point of using the governing principles as a simple predictor to avoid computationally-expensive simulation.

The work of this chapter suggests that the governing principles may be useful for high-resolution predictions in simpler models, but will not necessarily work well in more complex situations. They are still useful as a tool for understanding the large scale effects of changing fungicide application regimes (*e.g.* the effect of dose) but are not a replacement for simulation of more complex models.

6.5.2 Effects of the model of fungicide action

The results presented here concerning the effect of timing are in part explained by the fact that fungicide sprays applied before a leaf emerges provide no protection to that leaf. This

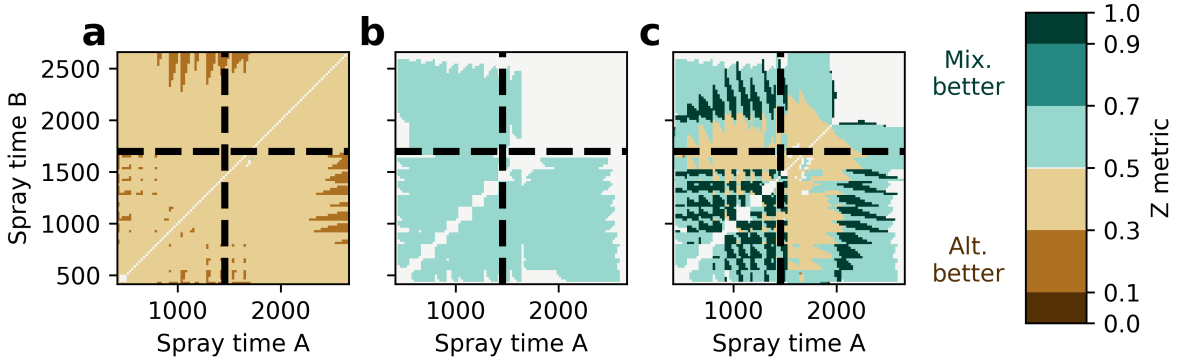

Figure 6.18: Relative performance of mixture and alternation when a full dose of both fungicides is applied and the timing of the sprays is varied. Tactics are compared according to **a)** selection ratio, **b)** first season yield and **c)** lifetime yield.

effect is accentuated by how fungicide action is modelled. The fungicide protectant action reduces susceptibility of healthy tissue and the eradicant action increases the length of the latent period. Neither of these effects target infectiousness directly, although the latent period effect does reduce it indirectly by reducing the amount of infectious tissue. If fungicides were modelled as reducing infectiousness, for example by reducing spore production, then the effect of spraying before leaf emergence would be expected to be reduced as the new leaf would receive some protection from the suppression of inoculum from lower layers.

The exact way in which fungicide effect is modelled likely had a bearing on the fact that the loss of protectant action with time did not seem to have a large effect on the results. In the current model, independent of the degree of infection already present on a given leaf the susceptible tissue remaining on that leaf receives the same level of protection. Critically, the reduction in the per capita rates of infection caused by the low-risk is unrelated to the severity of infection.

6.5.3 Model structure

It is notable that the impact of the particular timing of fungicide application was largely controlled by the specifics of the ElevenLeaf model, for example the particular area of each leaf layer at the time of application. This then raises the question of the accuracy of the specifics of this model. As discussed along with the specification of this model in chapter 2 there are a few aspects of the model that we believe may require refinement. Although certainly closer to reality than the FiveLeaf model, it would be sensible to devise some future experimental and modelling work in order to construct a more accurate model of crop development. It may be that the broad predictions are the same, as seen with the variety of models used in chapter 5, but this is not necessarily the case.

6.5.4 Optimising fungicide dose and timing

When a single spray of fungicide was applied at multiple times and doses, the performance of that spray (as measured by a number of metrics) was very variable. In particular, the optimal dose depended on the time of application and vice versa. This was also shown with a very similar model in van den Berg *et al.* (2013) and van den Berg *et al.* (2016). This highlights an interesting point, that recommendations for timing and dose should not be made independently.

The interaction between the fungicide dose-response parameters and spray timing was not investigated, but there is expected to be a similar interaction as with dose. The curvature parameter of the dose-response curve in fact has the same effect as dose in these models. Fungicide timings that are optimal for one fungicide are therefore not necessarily the same as for another. The effects of dose and expected effects of fungicide dose-response parameters imply that fungicide timing should be tailored to the exact use-case rather than using generic timings. This yet again highlights the need of accurate pathosystem-specific models.

When investigating the effect of timing and dose in a two spray regime, it was seen that there was a critical time period in which at least one spray should be applied in order to get the highest possible effective life. This critical time period includes the standard T2 timings; previous modelling work has also highlighted the importance of the T2 spray for maximising effective life (van den Berg *et al.*, 2016). The optimal tactic for two sprays of pyraclostrobin was found in our work to involve two applications of low dose during this critical period. This tactic was not identified in the previous modelling literature. It is possible that this tactic was not found before due to structural differences between the model used here and those in the literature. Alternatively it may be that this optimal tactic is dependent on the fungicide dose-response parameters, which are novel in our work. Finally it may not have been found previously simply because it was not tested. The first of the modern fungicide timing modelling papers examined shifting T1 and T2 sprays by a few weeks (van den Berg *et al.*, 2013), and the second examined the effect of the inclusion of different sprays at standard timings (T0 - T3) in a fungicide application regime (van den Berg *et al.*, 2016). Our optimal tactic is most similar to applying a late T2 and an early T3 spray, which is outside the range of tactics tested previously. It is also interesting that the optimal tactic involved such late sprays as standard advice is to apply fungicides earlier in epidemic development rather than later, although as pointed out earlier there is no experimental evidence behind this.

6.5.5 Implications of spray timing for mixture and alternation

We saw that when spray timings were variable and uncontrollable it was very rarely optimal to use alternation (figure 6.13). This shows that our earlier result of a mixture of as much low-risk as possible and just a little more high-risk than needed for initial control being optimal is largely invariant to the exact timing of the fungicide sprays. Therefore it is expected that a

similar tactic will be effective independent of the exact timings used, and probably also for regimes involving a greater number of sprays.

We also saw that allowing spray timing to be optimised for the chosen doses had a large effect on the pattern of relative strategy performance for mixture and alternation in dose-space. As stated before this is an unrealistic situation and has little practical value, but does highlight the complexity of the system.

Chapter 6 Summary

- The timing of a fungicide spray has a large effect on the selection pressure imposed and yield produced.
- The governing principles can explain the effect of spray timing in the FiveLeaf model.
- The governing principles are more difficult to use in the ElevenLeaf model, and their predictive power is weakened by the presence of a number of confounding mechanisms.
- The timing and dose of a fungicide spray cannot be optimised separately.
- The optimal timing for a fungicide spray depends on the doses and timings of other sprays.
- For a very small selection of non-standard T1 and T2 times it can be better for lifetime yield to use alternation rather than mixture.
- It is almost always better to use mixture rather than alternation with two sprays of fungicide, independent of the timing of the sprays.

7

Discussion

> *Nothing in biology makes sense except in the light of evolution.*
>
> Theodosius Dobzhansky, 1973

7.1 Summary

In this thesis we have used mathematical models to investigate the interaction between the way in which fungicides are applied and the evolution of fungicide resistance. In particular we have focussed on the use of alternation and mixture of two fungicides, and to a lesser extent the timing of application. In each case we have aimed not only to determine which fungicide application tactics are superior, but also to elucidate the mechanisms driving the differential performance of the tactics.

7.1.1 Mixture and alternation

In chapter 3 we compared mixture and alternation of a single high-risk and low-risk fungicide in a number of different ways, using two foliar applications per season to control septoria leaf blotch on winter wheat as the motivating example. Firstly, we fixed the total amount of fungicide applied under the two tactics to be the same. With this constraint we found that, depending on the dose of each fungicide, either tactic could perform better both in terms of resistance management alone and long-term yield production. For resistance management, alternation tended to perform better at higher doses of the high-risk and mixture at higher doses of the low-risk. For long-term yield mixture tended to perform better at lower doses of either fungicide. These results were explained in terms of dose-splitting, suppression of the resistant strain by the low-risk and the relationship between control and selection.

Given the clear relationship between control and selection, and the fact that mixture tends to impose more control for any given dose of fungicide compared to alternation due to dose-splitting, we then removed the constraint that each tactic must apply the same total amount of fungicide. We then saw that mixture was capable of producing a greater range of outcomes for both resistance management and yield compared to alternation. More importantly, the overall optimal tactic was to apply a mixture of as much low-risk as possible and just a little more high-risk than needed for initial disease control.

The fact that either mixture or alternation could prove better for resistance management for a fixed total dose matches the findings of the existing modelling and experimental literature, which show a variable pattern of which is better (van den Bosch *et al.*, 2014a). It is more difficult to relate our finding that the overall optimal tactic for lifetime yield was mixture to the literature. Experimental studies cannot be carried out over sufficiently long timescales to evaluate such metrics, and variation in individual grower practices means that extracting evidence for the impact of alternation and mixture from historical observations is difficult. It is only relatively recently that metrics related to yield have been considered in the fungicide resistance modelling literature (van den Bosch & Gilligan, 2008), and the only paper comparing mixture and alternation directly with a similar metric used a model almost identical to our FiveLeaf model (Hobbelen *et al.*, 2013). From the findings of this chapter alone, it is therefore not clear whether mixture is generally optimal or only in the chosen model of the wheat septoria pathosystem.

7.1.2 Robustness of the mixture and alternation comparison

To tackle the question of the generality of our main results, in chapter 4 we next took the comparisons and conclusions from chapter 3 and tested their robustness to the following factors.

- The values of key fungicide and epidemiological parameters.
- Modelling the powdery mildew of grape pathosystem instead of wheat septoria.
- Using a high-risk mixing partner instead of a low-risk.
- Partial instead of full resistance.

The main patterns in dose-space observed in chapter 3 were seen in all cases, but two cases lead to a change in the optimal tactic. Adding resistance to the mixing partner meant that it was no longer optimal to use as high a dose of the mixing partner as possible, however using a mixture was still optimal. When resistance was Type 2 partial (affecting the curvature parameter of the dose-response curve) and the degree of resistance was low, then it was optimal to apply more high-risk due to convergence of sensitive and resistant dose-response curves at higher dose, and to do so under alternation.

That the optimal tactic was largely unaffected by the exact construction or parameterisation of the model is strong evidence toward a general prediction that mixture will out-perform alternation in general. When resistance to the mixing partner was added, it was still optimal to use a large dose of that mixing partner. This is because the mixing partner was much less effective than the primary fungicide, and was in essence “sacrificed” in order to preserve the primary fungicide. This explanation fits with the findings of Hobbelen *et al.* (2013) in which they combine equally effective high-risk fungicides under mixture and alternation in a

very similar model, and show that the best effective lives are produced by a mixture with an equally low dose of each. Although not tackled in this chapter, a “rule of thumb” could likely be developed for mixture proportions in practice by considering the relative efficacy of the individual components.

Hobbelen *et al.* (2013) also examined the effect of partial resistance when mixing and alternating two high-risk fungicides. They did not find a case where partial resistance led to alternation being favoured over mixture however. This is either due to the differences in the models used, or more likely due to the degree of resistance investigated. We only observed alternation out-performing mixture when the resistant strain only had a small degree of resistance to the high-risk fungicide, the resistance degree r being on a scale of 0.25. In Hobbelen *et al.* (2013) the lowest resistance degree investigated was around 0.69.

The model of grapevine powdery mildew used is the only mathematical model for fungicide resistance in this pathosystem that we are aware of. To be able to generate generally effective tactics, it is important to ensure that mathematical models of a range of pathosystems are used, to make sure that the specifics of particular pathosystems are not conflated with general trends. Although the pathosystem modelled was changed, the core of the model was still very similar. In our later work we see that more complex host tissue dynamics (the ElevenLeaf septoria model) can introduce a number of counterintuitive effects, that make reasoning about such models difficult. It would be sensible therefore to produce a more realistic powdery mildew model, drawing on more of the complexity from the original simulation model (Calonnec *et al.*, 2008) that the simpler ODE models we built on (Burie *et al.*, 2011; Mammeri *et al.*, 2014) were designed to match.

7.1.3 The effect of model structure

By way of the powdery mildew case study, the main conclusions of the FiveLeaf model were seen to be relatively insensitive to the exact structure of the model in chapter 4. This was examined further in chapter 5 by constructing a set of models of complexity varying from a simple analytical model up to the full FiveLeaf model, to examine how the set of epidemiological mechanisms included in a model affect conclusions for resistance. Furthermore, the governing principles of fungicide resistance (van den Bosch *et al.*, 2014a) were used to explain the previous results in terms of a trade-off between dose-splitting and suppression of the resistant strain by the mixing partner. The trade-off predicted by the governing principles were put to a more rigorous test by formalising them using a simple analytical model and examining to what extent they could explain the results of the previous chapters. By examining the results of the sub-model models we isolated a number of features of the more complex models that can lead to a deviation from the expected trade-off.

Despite the identified mechanisms by which the models can deviate from the predictions of the governing principles, the principles still predicted the patterns observed in tactic

performance for resistance management with dose across all models with relatively high accuracy. The governing principles have already been shown to match well with the broad qualitative conclusions of experimental and modelling work (van den Bosch *et al.*, 2014a), but we believe this work represents the first test of their ability to predict tactic performance at higher resolution. The good predictive ability of the governing principles supports the practice in more recent fungicide resistance modelling papers of using them to explain observed results (Jørgensen *et al.*, 2017; van den Berg *et al.*, 2016; van den Bosch *et al.*, 2011, 2014b). However the existence of mechanisms that can have meaningful effects on the performance of fungicide application tactics, and are not predictable by the governing principles, shows that some care must be taken when applying the principles.

As well as investigating the effect of simplifying the model, we also used the more complex ElevenLeaf model. The detail of the predictions changed, but the broad patterns remained the same. This is consistent with our conclusion that the governing principles and simpler models are useful as a tool of understanding and for determining broad-stroke patterns, but are not sufficient for making detailed predictions about specific cases.

7.1.4 Spray timing

In the final chapter of research we investigated the effect of spray timing on the impact of fungicide applications. We started by investigating the effect of timing in the FiveLeaf model, in order to explain the differences between the two alternation tactics seen in the previous chapters. We showed that the effect of timing in that model is easily described through the governing principles.

We then investigated how the timing of a single spray affected its impact on selection and yield in the more complex ElevenLeaf model. We found that the structure of the model is such that timing has a large effect on the impact of a spray, but in such a way that it is not easily predictable using the governing principles. We also demonstrated that in both the FiveLeaf and ElevenLeaf models more control does not necessarily imply more selection, even without the use of a low-risk fungicide. Having used manipulation of a single spray to gain an understanding of the system, we then investigated how timing interacted with dose, mixture and alternation of two fungicide sprays.

When applying two sprays of fungicide, there was a critical span of time within which one spray had to be applied to get the greatest possible effective life. This critical time span includes the standard T2 timing, fitting with the results of van den Berg *et al.* (2016) that a T2 spray was integral to maximising effective life. We also demonstrated a clear interaction between timing and dose, such that one could not be optimised without taking the other into account. Ultimately the work presented on two sprays of a high-risk fungicide was very similar to that in van den Berg *et al.* (2013) and van den Berg *et al.* (2016), but we considered a wider range of application times and used a model with a more realistic treatment of lower

leaf layers.

The final part of this chapter was to examine the effect of varied spray timing on the relative performance of mixture and alternation of a high-risk and low-risk fungicide. We saw that changing the time of the two sprays only very rarely led to a case where alternation was preferred over mixture for maximising lifetime yield. This is useful as it further demonstrates the generality of mixture out-performing alternation and shows that unavoidable alterations in spray time in practice, for example due to weather, are unlikely to change the optimal tactic. Of course the optimal doses will depend on the exact spray time, but these are easier to modulate at the time of spraying rather than the use of mixture or alternation.

7.2 Summary of the major contributions of this thesis

The majority of the work in thesis was carried out to consider in detail the decades-old question of whether fungicides should be applied under mixture or alternation. We have concentrated almost exclusively (although see section 4.4) on the case of protecting a high-risk fungicide by mixing or alternating with a fungicide experiencing only a low risk of resistance development. Despite many experimental and modelling studies focussing on precisely this issue, no conclusive answer had as yet emerged to the important but very simple question of which provides better resistance management.

We have shown how whether mixture or alternation is the better strategy depends on precisely how the comparison is made. When considering only the degree to which each tactic selects for fungicide resistance, which is superior depends on the precise doses of fungicide applied, the parameterisation of the underlying epidemiological model and the details of the dose-response curves of the fungicides. However any realistic spray programme must also lead to sufficient disease control. When this is taken into account, by considering the yield over the effective lifetime of the fungicide in which the yield remains above an economic threshold, we find that in the overwhelming majority of cases mixtures out-perform alternation (chapters 3, 4).

The need to balance the selection pressure imposed by a spray programme against its effect on disease control has been highlighted for some time (van den Bosch & Gilligan, 2008). Indeed a number of recent papers have concentrated on testing the implications of this idea, using almost exactly the same models of the wheat-septoria pathosystem as used here (*e.g.* Hobbelen *et al.* (2013); Kitchen *et al.* (2016); van den Berg *et al.* (2013)). One of these studies showed a similar result to our own, that mixtures are optimal compared to alternation (Hobbelen *et al.*, 2013).

The key distinguishing feature of our work, however, is our sustained focus on examining potential barriers to mixture out-performing alternation. By comparing results across models and model parameterisations, we tested the robustness of the result to a range of epidemiological parameters (section 4.2), as well as to the particular pathosystem that is modelled

(section 4.3) and timing of sprays (section 6.4). We also tested, via our sensitivity analysis to model structure, how the set of epidemiological mechanisms included in the underlying model and the detail of how host tissue growth is modelled affect our conclusions (chapter 5).

Notably, we found the optimum tactic was independent of all of these factors. We are not aware of any other studies in the fungicide modelling literature, or even the plant disease modelling literature more generally, performing a similarly extensive comparison. We therefore feel justified in now saying that it will be optimal to apply a full dose of low-risk mixed with just a little more high-risk than required for minimal effective control, and that this should be applied as a mixture.

The other key aspect distinguishing our approach from the existing literature is our focus on understanding the drivers of the results. The recent formalisation by van den Bosch *et al.* (2014a) of the governing principles originally identified by Milgroom and Fry provides a powerful theoretical framework to understand how a given spray programme affects selection for fungicide resistance. Here we have tested the extent to which the governing principles can be used an underpinning theory. In general we have found that the governing principles can largely explain the impact of a spray regime on selection, although in some cases some ingenuity is required to see how the governing principles should correctly be applied. We have also shown how the governing principles can be used to understand, in some detail, the effect of spray timing on the development of resistance (chapter 6). However, we have also demonstrated situations within which the governing principles alone are not sufficient to predict the outcome, highlighting that detailed system-specific models are required (*e.g.* the timing of fungicide sprays in the detailed ElevenLeaf model, section 6.3). It is our focus on testing the limits of applicability of our results, as well as showing how underpinning epidemiological driving mechanisms can be identified and quantified via the governing principles, that is the major contribution of this thesis.

7.3 Relevance of work to agronomic practice

The main conclusion of our work with direct pertinence to agronomic practice is the observation that, independent of a large number of different factors, the optimal fungicide application tactic is to apply a mixture of as much low-risk fungicide as possible and a little more high-risk than needed for initial control. Mixture is already used commonly in practice, with some products sold as pre-mixed solutions and others specifying on their label that they must only be used in tank mixture. As well as their anti-resistance benefit, mixture is also used to increase the range of diseases controlled and as a security measure should resistance lead to failure of one component of the mixture (van den Bosch *et al.*, 2014b). However it is very notable that FRAC in general recommends that the doses in mixture be chosen such that either component could provide adequate control alone (Fungicide Resistance

Action Committee, 2010). Our results agree with the use of mixture, but would suggest that doses should be reduced. This may in part be due to the relative simplicity of our economic analysis, where fungicides are simply used until eventual inevitable failure of control. We do not consider risk aversion, which is likely the cause of the FRAC dose recommendations. It is possible that the doses used under FRAC guidelines are high enough that alternation may be a better choice, but as shown this will depend on the exact pathosystem and fungicides.

On possible challenge to application of these findings is that the optimal tactic involves precise choice of dose. The resolution to which growers can manipulate the dose applied may well be limited by the machinery used, and as explored in Shaw (2000) the actual dose experienced by any given fungal lesion will vary. If for example, a grower can only control dose to the nearest quarter dose then the nearest lower quarter dose to the optimal may not provide sufficient control and the nearest higher quarter dose may be high enough a dose that mixture is no longer preferable. The size of the area in dose-space for which mixture out-performs alternation is fortunately in the cases examined large enough that even the conservative estimate of quarter dose resolution does not lead to alternation being preferable. The question of dose heterogeneity is considered to some degree in our work with the ElevenLeaf model, due to the differential interception of dose by each leaf. Although dose heterogeneity has important effects on the performance of the application tactics, it does not stop mixture from being overall optimal.

Research into fungicide resistance management tactics is particularly timely currently, as the upcoming exit of the United Kingdom from the European Union may provide an opportunity for an evidence-based re-evaluation of fungicide policy in the UK.

7.4 Application to other pesticides

We have shown that our main conclusions are, at least in part, insensitive to the exact pathosystem investigated. One might ask therefore whether these results can be used to inform tactics to control resistance development to other non-fungicidal pesticides. In the fields examining resistance to many other pesticides the commonly-held wisdom is that increased doses suppress resistance development, which is directly opposed to the conclusions of the experimental and modelling work in fungicides (van den Bosch *et al.*, 2011, 2014a). The reasons for this difference are discussed in section 1.8. Since the optimal tactic is in general decided by the optimal dose of the high-risk (*cf.* partial and full resistance results), mixtures may not perform as well in these other cases. However, this does not necessarily mean that we would expect alternation to perform better as there are features in these pathosystems not present in fungal diseases that may cause significant differences, such as the diploidy of insects or the long-lived seed bank of weeds.

The most similar case is the use of antibiotics to target bacterial disease in agriculture. It seems likely that our models would be applicable to this case, and thus might lead to

similar conclusions. The use of antibiotics in humans is however unlikely to show the same dynamics as treatment efficacy is much more important in that case compared to agriculture, where reasonable losses to disease are acceptable.

Even if the conclusions of analyses may vary between pesticides, the techniques applied in this thesis are broadly applicable. For example Carolan *et al.* (2017) made use of the governing principles to investigate the evolution of pathogen traits overcoming host resistance.

7.5 Potential improvements and future direction

With the benefit of hindsight, there are a number of improvements to the presented work that could be done "if we had our time again". These improvements often point clearly to future directions for research in this area.

7.5.1 Data-driven models

One of the key issues we experienced throughout the course of the work for this thesis was a difficulty in obtaining suitable data for fitting models. A number of assumptions were made in order to construct the mathematical models used here. Many of these assumptions are made commonly in the literature, and yet it is difficult to determine what data or observations, if any, underlie some of them. The main sources of data used to parameterise our models were reports from the HGCA. There are two main difficulties in using these data with the models presented: the data are averages and the data recorded cannot be used as direct input into the models.

In general the fungicide efficacy data available represent average values calculated across multiple trial sites and sometimes years. This means that any variance due to confounding factors such as weather or other agronomic practices can not be excluded by any further analysis. If data were presented for each trial separately, then these factors could be accounted for when using the data. For example when fitting fungicide parameters, fits could be carried out for individual epidemics with the force of infection allowed to vary between fits.

The typical quantities recorded during trials are values such as the yield produced by the crop, or the disease severity at a given time. While these quantities are valuable, they can not be used to directly parameterise the dose-response curves used in our models. Fitting techniques must instead be used to match the output of the models to trial data in an attempt to infer the parameters controlling the shape of the dose-response curves. These techniques can be computationally expensive, and issues with identifiability or "poorly-behaved" objective functions can cause them to produce inappropriate results. Ideally data closer to the effects the dose-response curves are used to model would be available, such

as direct measurements of decreases in lesion spore production when treated with fungicide. Sometimes this kind of data is available under laboratory conditions, but it is not clear how well these translate to field conditions. The difficulty in fitting these models is not restricted to fungicide parameters, but also the more general epidemic parameters.

Data confidentiality presents a further impediment to getting access to what data exists, as desirable data is often used as evidence of fungicide efficacy. This evidence is key to registration of new products, and represents a significant investment by agrochemical companies. Without close industry connections, it is therefore very difficult to get access to the data that would be able to either verify or improve the type of models used in this thesis.

7.5.2 Dose-response curves

One of the key assumptions made in the models used is that the protectant and eradicant dose-response curves were the same for fungicides that have both of these effects. This is very unlikely to be true in practice. For example van den Berg *et al.* (2013) states that the fungicide they investigate is equally effective as a protectant and eradicant, and use this as justification for giving the same dose-response curve to each effect. But the statement of equality of effect comes from looking at severity-dose curves, and applying the same proportional reduction to different epidemiological parameters will not in general lead to the same reduction in disease severity. Therefore a fungicide showing the same protectant and eradicant activity in the field would actually imply that that the dose-response curves for the effects on the infection rate and latent period should be different. van den Berg *et al.* (2013) is not alone in making this assumption, but is particularly notable as they emphasise the difference between the dose-response curves uses in field trials and those used in the model. There is good experimental evidence for fungicides having different dose-response curves for their different effects, for example trifloxystrobin and quinoxyfen have similar suppressive effects on mycelial development of *Erysiphe necator* but quinoxyfen has a much smaller effect on spore production (Deliere *et al.*, 2010).

As well as the assumption of equality of the dose-response curves, the parameters for the dose-response curves are generally fit to very little or poor data. The justification can be made that it is just important to get a curve close to reality rather than a very specific fit, as the underlying parameters themselves lack biological meaning and instead just represent a parsimonious mathematical approximation. However, our analyses have shown that the different parameters of the dose-response curve can have different effects on the predictions of models. For example, exponential dose-response curves that are very similar over the range of legal doses can be produced by increasing one parameter and decreasing the other. The maximum effect parameter for the high-risk fungicide was shown to have no effect on the relative performance of mixture and alternation whilst the curvature parameter was important. Attempting to reconcile this with the lack of identifiability when fitting these

parameters to severity data raises some interesting questions.

The exact way in which fungicides affect pathogens is generally not given much consideration in mathematical models. This is particularly interesting as there is evidence that fungicides showing very similar severity dose-response cures can show different dynamics with regard to resistance. For example fosetyl-aluminium and mancozeb were shown to have a similar suppressive effect, through different modes of actions, on *Plasmopara viticola* but the former was more effective at retarding QoI resistance development when used as a mixing partner (Genet *et al.*, 2006). In addition, the data on fungicide performance on wheat provided by the HGCA makes it clear that resistance can affect protectant and eradicant activity differently. For example over the same period that protectant control for Proline dropped from around 90% to around 70%, its eradicant control dropped from around 80% to around 20% (Home Grown Cereals Authority, 2014). The resistant strain may be better modelled in that case by having different degrees of resistance on the eradicant and protectant dose-response curves, although the relationship between protectant and eradicant dose-response curves from severity data and those used in the models in this thesis are not clear. Furthermore it has become typical in the modern literature to assume that eradicants increase the length of the latent period. Earlier works however considered, perhaps more intuitively, that fungicides might lead to the death of lesions (*e.g.* Josepovits (1989); Shaw (2006)). It is not clear what experimental data has led to the modern assumption of eradicant action only increasing the latent period.

In the most modern set of fungicide resistance models, each individual leaf layer is modelled separately (Kitchen *et al.*, 2016; van den Berg *et al.*, 2013, 2016). In models with homogeneous mixing of host tissues a reduction in infectiousness has the same effect as a reduction in susceptibility, but this is not the case when spatial structure is introduced such as in these models. It is therefore interesting that protectant activity has in each case been modelled as a reduction in susceptibility, with no mention of reductions in infectiousness. This is particularly interesting as at least in one case data on the reduction in infectiousness was used to parameterise dose-response curves for the reduction in susceptibility (Kitchen *et al.*, 2016).

These assumptions clearly lend themselves to two investigations. Firstly a theoretical study could be carried out to investigate what impact these assumptions have on the predictions of resistance models. Secondly a data-driven study could be carried out to determine how well these assumptions match reality, and whether alternative assumptions might be more realistic.

7.5.3 Dose-response curves and effect independence

For our analysis of the effect of model structure on predictions, we chose features that differentiate a simple analytical model from the full models adapted from the literature. The

choice of these features was largely due to the fact that the analytical model was constructed as an early part of our work, and the chosen features were those that distinguished that simple model from the full models used later. Analysing the effect of these features was informative, but ultimately none of these features affected what is arguably the core of the model. Many of the mechanisms driving the results in the models come from the shape of exponential dose-response curves and way in which independence of effect in mixture is modelled. It would be interesting to examine the effect of changing these core components of the models on the predictions generated. In particular, it is important to know if our predictions about when mixture or alternation perform better are conditioned on the exact model of independence and the shape of the dose-response curve.

It is notable that a reasonably large part of the fungicide resistance modelling literature is concerned with ways of modelling independence of effect, providing a solid basis for further research. Shaw (1989b) developed a framework for considering independence of effect in exponential growth models, and explained why it is unlikely that fungicides would show additivity or multiplicativity of effect on the growth rate. Although this reasoning does not tell us anything about how fungicides are expected to affect individual lifecycle processes, the same framework for defining deviation from independence could be adapted.

7.5.4 More realistic treatment of pathogen strains

In one chapter we briefly touched upon the effect of adding strains resistant to the mixing partner, and also partial resistance, on the performance of mixture and alternation. Both of these effects were capable of leading to a situation where the optimal tactic was not necessarily to apply a mixture of as much of the mixing partner as possible and just slightly more of the primary fungicide than needed for initial disease control. This therefore suggests that accurately modelling the pathogen strains, and their level of resistance to particular fungicides, accurately is important. The assumption of two strains with full resistance and full sensitivity eases analysis, but is likely an oversimplification. For example, resistance is in some cases better described as a “sensitivity shift” rather than binary resistance or susceptibility, for example DMI sensitivity in powdery mildew of grape shows a continuous distribution (Milgroom, 2015). Indeed the fact that resistance is generally described as a resistance factor, the multiple by which the dose has to be increased to get the same effect on the resistant strain as the sensitive, shows that full resistance is biologically unrealistic. A sufficiently large dose of almost anything is likely to be toxic.

There are only very few fungicide resistance modelling papers considering polygenic resistance (Birch & Shaw, 1997; Shaw, 1989a, 2000). Each of these papers uses relatively simple models, although it is demonstrated that the results are independent of a number of the simplifying assumptions made. However the consideration of host tissue dynamics is minimal; it would be interesting to combine these models with one or more of the host tissue

models used in this thesis.

A further complexity that has not been considered in our analyses is cross-resistance. Positive cross-resistance will reduce the effectiveness of both mixture and alternation, but it is not clear what level of cross-resistance would be required to make these strategies effectively the same as solo application. Indeed, it is possible that cross-resistance, either positive or negative, may affect mixture and alternation to differing degrees and so could change the conclusion of which is better.

Considering negative cross-resistance also opens up the consideration of other strategies, such as the "Merry Dance" of Oliver (2016), which could be compared to mixture and alternation. This refers to using one fungicide exclusively for a time, increasing resistance to that fungicide but also increasing sensitivity to a second fungicide through negative cross-resistance, and then switching to using the second fungicide exclusively. In an ideal world this tactic allows perpetual effective use of both fungicides, as long as they are switched at the appropriate times and the appropriate cross-resistance exists. However it is not clear how realistic this ideal is. Patterns of cross-resistance between fungicides are complex; depending on the exact resistance-granting mutations, fungal species and fungicides involved (Sierotzki & Scalliet, 2013). Furthermore, interactions leading to negative cross-resistance may only be transient in the population. As an example, a carendazim diethofencarb mixture was applied to take advantage of the negative cross-resistance present in the BenR1 phenotype of French vineyard *Botrytis cinerea*. On the introduction of the new mixture the BenR1 phenotype was replaced in the fungal population by the BenR2 phenotype which shows positive cross-resistance for these fungicides (Hollomon, 2015).

7.5.5 Risk aversion and environmental stochasticity

The concept of risk aversion has been raised previously, the idea that a grower can not be sure of exactly how high disease pressure is likely to be in any given season and may choose to apply stronger control than strictly necessarily for the average epidemic to avoid catastrophic failure under an extreme epidemic. All of the models we have considered are deterministic, and every season is identical apart from the build-up of the resistant strain. By adding environmental stochasticity into the models, we could investigate the effect of risk aversion on the optimal fungicide application tactic. This could have a significant effect as in general the optimal tactics we have identified have relied on applying doses that skirt failure of control. Increasing doses would be likely to favour alternation, however the in-built insurance mechanisms in mixture may out-weigh this.

Taking environmental stochasticity into account with fungicide applications has been modelled before (te Beest *et al.*, 2013). In that paper a distribution of seasonal disease severities was fed into a very simple economic and epidemiological model in order to determine how risk aversion affected the optimal fungicide tactic. Risk averse tactics were

identified by minimising costs, yield loss to disease and fungicide expenditure, given the constraint that a yield loss over a certain threshold could be only be tolerated at a certain frequency. A similar approach could be used with our models, varying the base disease severity each season according to a distribution matched to data. Disease severity could be modulated by altering pathogen lifecycle rates (*e.g.* the infection rate parameter) or the amount of primary inoculum. Interesting theoretical questions about how disease pressure is modelled could be tackled as well as the applied question of how risk aversion affects fungicide anti-resistance tactics.

7.5.6 Other pathosystems

There is a very clear trend in the modern fungicide resistance modelling literature of using septoria on UK winter wheat as the case pathosystem (Hobbelen *et al.*, 2011a, 2013, 2014; Mikaberidze *et al.*, 2017; van den Berg *et al.*, 2016). This is likely due to the importance of septoria in Western European arable agriculture and the availability of data on this pathosystem. While these highly specialised models can give high quality information about the impact of fungicide application tactics in this specific pathosystem, they cannot tell us if these results are general. For example, van den Bosch *et al.* (2014b) states that mixture may not work well for monocyclic pathogens and van den Berg *et al.* (2013) points out that powdery mildew of wheat shows a greater degree of aerial dispersal leading to more homogeneous spore spread across the canopy. We have attempted to account for this in our work by comparing the predictions of specific models to models of other pathosystems and simpler more general models. However, this it is important to continue to confirm the generality of other findings from the literature against other pathosystems.

7.5.7 Multiple pathogens

Similar to the idea of generality of models is that fungicide treatments are not optimised with a single disease in mind; in reality a grower applies fungicides to protect the crop from a variety of pathogens. If fungicide resistance in different pathogens is best managed in different ways, then there is a trade-off to be investigated. For example, is it better to focus on controlling resistance in the most damaging pathogens only or to attempt to manage resistance in all pathogens to some extent? Considering the threat from multiple pathogens at once is not typical in the wider epidemiological modelling literature, but represents a key challenge for the future (Cunniffe *et al.*, 2015b). Research in this area relies on the existence of good quality models of a number of diseases for a single crop and so currently is untenable, but should be considered a future aim for increasing the applicability of theoretical findings.

7.5.8 More complex strategies

Throughout this thesis the treatment of mixture and alternation tactics has been relatively simple, assuming that the doses applied each season are the same and that there are exactly two sprays of fungicide each season. However fungicide application strategies need not be static over time; for example Hobbelen *et al.* (2011a) considered the dose of a high-risk fungicide being increased over time to account for reduced effectiveness. This would increase the lifetime of both alternation and mixture, and would potentially favour mixture further by permitting the use of even lower doses. Alternation and mixture as presented here represent two ends of a spectrum. There are a range of tactics between that could be examined, for example an early spray with a mixture consisting mainly of low-risk and a later of one consisting mainly of high-risk. There is much a larger volume of tactic-space that could be considered in future work.

We have assumed that there are only a single pair of fungicides available for use, whereas for particular applications there may be more. This further increases the range of possible mixture and alternation tactics that could be considered. Increasing the number of fungicides used would be expected to increase the effective lifetime of each fungicide, but it is not clear if the performance of mixture and alternation will scale in the same way with the number of fungicides.

With the increased computational power available today, the wider epidemiological literature is beginning to consider complex spatial patterns of disease control (Cunniffe *et al.*, 2015a, 2016; Gilligan *et al.*, 2007; Hyatt-Twynam *et al.*, 2017; Parnell *et al.*, 2010; Tildesley *et al.*, 2006). In the same way anti-resistance strategies that rely on spatial structuring of fungicide sprays may be effective. A few modelling papers have been published on the spatial aspects of fungicide resistance but in a relatively simplistic fashion (Hobbelen *et al.*, 2013; Parnell *et al.*, 2005, 2006).

7.6 Concluding remarks

In this thesis we have investigated the dynamics of fungicide resistance evolution in response to the particular patterns of fungicide application. We have provided strong evidence to support the idea that when using a low-risk and high-risk fungicide, mixture will very often out-perform alternation. However the fact that we cannot prove that this is true in all cases, or identify clear conditions for when it is true, highlights the complex nature of fungicide resistance evolution. There is still much work to do to produce equally strong evidence in support of other key agronomic choices, for example the timing of fungicide sprays.

We have built on a body of work that spans many decades. Despite the large amount of effort expended on understanding this topic, there is clearly much that still remains unknown. Some of the unknown features of the system are perhaps surprisingly fundamental, for

example the questions raised about the particulars of the models in the second chapter. It is important that future work attempts to resolve some of these basic questions, so that the foundations of the work on more complex questions can be verified.

Research into fungicide resistance is important now and will only become more important. Resistance is a problem that will get worse with time, and the food production system is also under increasingly greater pressure from a variety of other factors. While viable alternatives to fungicidal control may become available in the future, for the time being they are a central part of feeding the growing world population, and must be preserved.

Bibliography

Amaradasa, B.S., & Everhart, S.E. 2016. Effects of sublethal fungicides on mutation rates and genomic variation in fungal plant pathogen, *Sclerotinia sclerotiorum*. *PLoS ONE*, **11**, e0168079.

Avenot, H.F., & Michailides, T.J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Protection*, **29**, 643–651.

Berenbaum, M.C. 1989. What is synergy? *Pharmacological Reviews*, **41**, 93–141.

Birch, C.P.D., & Shaw, M.W. 1997. When can reduced doses and pesticide mixtures delay the build-up of pesticide resistance? A mathematical model. *Journal of Applied Ecology*, **34**, 1032–1042.

Brent, K.J., & Hollomon, D.W. 2007a. *Fungicide Resistance in Crop Pathogens: How Can it be Managed?* Second rev edn. FRAC.

Brent, K.J., & Hollomon, D.W. 2007b. *Fungicide Resistance: The Assessment of Risk*. Second rev edn. FRAC.

Brewer, M.T., Frenkel, O., & Milgroom, M.G. 2012. Linkage disequilibrium and spatial aggregation of genotypes in sexually reproducing populations of *Erysiphe necator*. *Phytopathology*, **102**, 997–1005.

Burie, J., Langlais, M., & Calonnec, A. 2011. Switching from a mechanistic model to a continuous model to study at different scales the effect of vine growth on the dynamic of a powdery mildew epidemic. *Annals of Botany*, **107**, 885–895.

Calonnec, A., Cartolar, P., Poupot, C., Dubourdieu, D., & Darriet, P. 2004. Effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) and wine. *Plant Pathology*, **53**, 434–445.

Calonnec, A., Cartolaro, P., Delière, L., & Chadoeuf, J. 2006. Powdery mildew on grapevine: the date of primary contamination affects disease development on leaves and damage on grape. *IOBC/WPRS Bulletin*, **29**, 67–73.

Calonnec, A., Cartolaro, P., Naulin, J.-M., Bailey, D., & Langlais, M. 2008. A host-pathogen simulation model: powdery mildew of grapevine. *Plant Pathology*, **57**, 493–508.

Calonnec, A., Wiedemann-Merdinoglu, S., Delière, L., Cartolaro, P., Schneider, C., & Delmotte, F. 2013. The reliability of leaf bioassays for predicting disease resistance on

fruit: a case study on grapevine resistance to downy and powdery mildew. *Plant Pathology*, **62**, 533–544.

Carolan, K., Helps, J., van den Berg, F., Bain, R., Paveley, N., & van den Bosch, F. 2017. Extending the durability of cultivar resistance by limiting epidemic growth rates. *Proceedings of the Royal Society, B*, **284**, 20170828.

Chin, K.M., Chavaillaz, D., Kaesbohrer, M., Staub, T., & Felsenstein, F.G. 2001. Characterizing resistance risk of *Erysiphe graminis* f.sp. *tritici* to strobilurins. *Crop Protection*, **20**, 87–96.

Cohen, Y, & Levy, Y. 1990. Joint action of fungicides in mixtures: theory and practice. *Phytoparasitica*, **18**, 159–169.

Cortesi, P., Ottaviani, M., & Milgroom, M.G. 2004. Spatial and Genetic Analysis of a Flag Shoot Subpopulation of *Erysiphe necator* in Italy. *Phytopathology*, **94**(6), 544–550.

Crow, J.F., & Kimura, M. 1970. *An Introduction To Population Genetics Theory*. Harper and Row.

Cunniffe, N.J., Stutt, R.O.J.H., van den Bosch, F., & Gilligan, C.A. 2012. Time-dependent infectivity and flexible latent and infectious periods in compartmental models of plant disease. *Phytopathology*, **102**, 365–380.

Cunniffe, N.J., Stutt, R.O.J.H., DeSimone, R.E., Gottwald, T.R., & Gilligan, C.A. 2015a. Optimising and communicating options for the control of invasive plant disease when there is epidemiological uncertainty. *PLoS Computational Biology*, **11**, e1004211.

Cunniffe, N.J., Koskella, B., Metcalf, C.J.E., Parnell, S., Gottwald, T.R., & Gilligan, C.A. 2015b. Thirteen challenges in modelling plant diseases. *Epidemics*, **10**, 6–10.

Cunniffe, N.J., Cobb, R.C., Meentemeyer, R.K., Rizzo, D.M., & Gilligan, C.A. 2016. Modelling when, where and how to manage a forest epidemic, motivated by sudden oak death in California. *Proceedings of the National Academy of Sciences*, **113**, 5640–5645.

Day, T., & Read, A.F. 2016. Does High-Dose Antimicrobial Chemotherapy Prevent the Evolution of Resistance? *PLoS Computational Biology*, **12**, e1004689.

Dekker, J. 1986. Preventing and Managing Fungicide Resistance. *Pages 347–54 of: Pesticide Resistance: Strategies and Tactics for Management*. Washington, DC: National Academy Press.

Deliere, L., Miclot, A.S., Sauris, P., Rey, P., & Calonnec, A. 2010. Efficacy of fungicides with various modes of action in controlling the early stages of an *Erysiphe necator*-induced epidemic. *Pest Management Science*, **66**, 1367–1373.

Delière, L., Cartolaro, P., Léger, B., & Naud, O. 2015. Field evaluation of an expertise-based formal decision system for fungicide management of grapevine downy and powdery mildews. *Pest Management Science*, **71**, 1247–1257.

Delp, C.J. 1980. Coping with resistance to plant disease control agents. *Plant Disease*, **64**, 652–657.

Di Veroli, G.Y., Fornari, C., Goldlust, I., Mills, G., Koh, S.B., Bramhall, J.L., Richards, F.M, & Jodrell, D.I. 2015. An automated fitting procedure and software for dose-response curves with multiphasic features. *Scientific Reports*, **5**, 14701.

Eriksen, L., Shaw, M. W., & Østergård, H. 2001. A model of the effect of pseudothecia on genetic recombination and epidemic development in populations of *Mycosphaerella graminicola*. *Phytopathology*, **91**(3), 240–248.

Fones, H., & Gurr, S. 2015. The impact of septoria tritici blotch disease on wheat: an EU perspective. *Fungal Genetics and Biology*, **79**, 3–7.

Fraaije, B.A., Butters, J.A., Coelho, J.M., Jones, D.R., & Hollomon, D.W. 2002. Following the dynamics of strobilurin resistance in *Blumeria graminis* f. sp. *tritici* using quantitative allele-specific real-time PCR measurements with the fluorescent dye SYBR Green I. *Plant Physiology*, **51**, 45–54.

Fraïsse, C., Elderfield, J. A. D., & Welch, J. J. 2014. The genetics of speciation: are complex incompatibilities easier to evolve? *Journal of Evolutionary Biology*, **27**, 688–699.

Fungicide Resistance Action Committee. 2010. *Recommendations for fungicide mixtures designed to delay resistance evolution*. http://www.frac.info/docs/default-source/publications/frac-recommendations-for-fungicide-mixtures/frac-recommendations-for-fungicide-mixtures—january-2010.pdf.

Fungicide Resistance Action Committee. 2017a. *Mechanisms of Fungicide Resistance*. http://www.frac.info/resistance-overview/mechanisms-of-fungicide-resistance.

Fungicide Resistance Action Committee. 2017b. *What is Resistance?* http://www.frac.info/resistance-overview.

Fungicide Resistance Management Committee. 2017. *SDHI Fungicides: Introduction and General Information*. http://www.frac.info/working-group/sdhi-fungicides/introduction-and-general-information.

Gadoury, D.M., Seem, R.C., Ficke, A., & Wilcox, W.F. 2003. Ontogenic resistance to powdery mildew in grape berries. *Phytopathology*, **93**, 547–555.

Gadoury, D.M., Cadle-Davidson, L., Wilcox, W.F., Dry, I.B., Seem, R.C., & Milgroom, M.G. 2012. Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Molecular Plant Pathology*, **13**, 1–16.

Geary, N. 2013. Understanding synergy. *American Journal of Physiology*, **304**, E237–253.

Genet, J.-L., Jaworska, G., & Deparis, F. 2006. Effect of dose rate and mixtures of fungicides on selection for QoI resistance in populations of *Plasmopara viticola*. *Pest Management Science*, **62**, 188–194.

Georgopoulos, S.G., & Zaracovitis, C. 1967. Tolerance of fungi to organic fungicides. *Annual Review of Phytopathology*, **5**, 109–130.

Gilligan, C.A., & van den Bosch, F. 2008. Epidemiological Models for Invasion and Persistence of Pathogens. *Annual Review of Phytopathology*, **46**, 385–418.

Gilligan, C.A., Truscott, J.E., & Stacey, A.J. 2007. Impact of scale on the effectiveness of disease control strategies for epidemics with cryptic infection in a dynamical landscape: an example for a crop disease. *Journal of the Royal Society Interface*, **4**, 925–934.

Gooding, M.J., & Dimmock, J. 2000. Green leaf area decline of wheat flag leaves: the influence of fungicides and relationships with mean grain weight and grain yield. *Annals of Applied Biology*, **136**, 77–84.

Gubbins, S, & Gilligan, C.A. 1999. Invasion thresholds for fungicide resistance: deterministic and stochastic analyses. *Proceedings of the Royal Society, B*, **266**, 2539–2549.

Gullino, M.L., & Kuijpers, L.A.M. 1994. Social and political implications of managing plant diseases with restricted fungicides in Europe. *Annual Reviews of Phytopathology*, **32**, 559–579.

Hall, R.J., Gubbins, S., & Gilligan, C.A. 2004. Invasion of drug and pesticide resistance is determined by a trade-off between treatment efficacy and relative fitness. *Bulletin of Mathematical Biology*, **66**, 825–840.

Hall, R.J., Gubbins, S., & Gilligan, C.A. 2007. Evaluating the performance of chemical control in the presence of resistant pathogens. *Bulletin of Mathematical Biology*, **69**, 525–537.

Hobbelen, P.H.F., Paveley, N.D., & van den Bosch, F. 2011a. Delaying selection for fungicide insensitivity by mixing fungicides at a low and high risk of resistance development: a modeling analysis. *Phytopathology*, **101**, 1224–1233.

Hobbelen, P.H.F., Paveley, N.D., Fraaije, B.A., Lucas, J.A., & van den Bosch, F. 2011b. Derivation and testing of a model to predict selection for fungicide resistance. *Plant Pathology*, **60**, 304–313.

Hobbelen, P.H.F., Paveley, N.D., Oliver, R.P., & van den Bosch, F. 2013. The usefulness of fungicide mixtures and alternation for delaying the selection for resistance in populations of *Mycosphaerella graminicola* on winter wheat: a modeling analysis. *Phytopathology*, **103**, 690–707.

Hobbelen, P.H.F., Paveley, N.D., & van den Bosch, F. 2014. The emergence of resistance to fungicides. *PLoS ONE*, **9**, e91910.

Hollomon, Derek W. 2015. Fungicide Resistance: Facing the Challenge. *Plant protection science*, **51**(4), 170–176.

Home Grown Cereals Authority. 2014. *Fungicide Performance in Wheat, 2014*. https://cereals.ahdb.org.uk/media/547811/Fungicide-performance-in-wheat-2014-V2.pdf.

Hyatt-Twynam, S.R., Parnell, S., Stutt, R.O.J.H., Gottwald, T.R., Gilligan, C.A., & Cunniffe, N.J. 2017. Risk-based management of invading plant disease. *New Phytologist*, **214**, 1317–1329.

Jørgensen, L.N., van den Bosch, F., Oliver, R.P., Heick, T.M., & Paveley, N. 2017. Targeting fungicide inputs according to need. *Annual Review of Phytopathology*, **55**, 181–203.

Josepovits, G. 1989. A model for evaluating factors affecting the development of insensitivity to fungicides. *Crop Protection*, **8**, 106–113.

Josepovits, G., & Dobrovolszky, A. 1985. A novel mathematical approach to the prevention of fungicide resistance. *Pesticide Science*, **16**, 17–22.

Jyot, G., Arora, P.K., Sahoo, S.K., Singh, B., & Battu, R.S. 2010. Persistence of Trifloxystrobin and Tebuconazole on grape leaves, grape berries and soil. *Bulletin of Environmental Contamination and Toxicology*, **84**, 305–310.

Kable, P.F., & Jeffery, H. 1980. Selection for tolerance in organisms exposed to sprays of biocide mixtures: a theoretical model. *Phytopathology*, **70**, 8–12.

Kermack, W.O., & McKendrick, A.G. 1927. A contribution to the mathematical theory of epidemics. *Proceedings of the Royal Society, A*, **115**, 700–721.

Kitchen, J.L., van den Bosch, F., Paveley, N.D., Helps, J., & van den Berg, F. 2016. The evolution of fungicide resistance resulting from combinations of foliar-acting systemic seed treatments and foliar-applied fungicides: a modeling analysis. *PLoS ONE*, **11**, e0161887.

Koller, W., & Wubben, J.P. 1988. Variable resistance factors of fungicides acting as sterol demethylation inhibitors. *Pesticide Science*, **26**, 133–145.

Leonard, K.J., & Fry, W.E. 1989. *Plant Disease Epidemiology 2: Genetics, Resistance and Management*. McGraw-Hill Education.

Levy, Y., & Levy, R.S. 1986. Control strategies using systemi fungicides for limiting disease development and resistance buildup: practical implications of a simulation model. *Phytoparasitica*, **14**, 303–312.

Levy, Y., Levi, R., & Cohen, Y. 1983. Buildup of a pathogen subpopulation resistant to a systemic fungicide under various control strategies: a flexible simulation model. *Phytopathology*, **73**, 1475–1480.

Löcher, F.J., Lorenz, G., & Beetz, K.J. 1987. Resistance management strategies for dicarboximide fungicides in grapes: results of six years' trial work. *Crop Protection*, **6**, 139–147.

Lockley, D., & Clark, W. S. 2005. *Fungicide dose-response trials in wheat: the basis for choosing "appropriate dose"*. Tech. rept.

Madden, L.V. 2006. Botanical epidemiology: some key advances and its continuing role in disease management. *European Journal of Plant Pathology*, **115**, 3–23.

Mammeri, Y., Burie, J.B., Langlais, M., & Calonnec, A. 2014. How changes in the dynamic of crop susceptibility and cultural practices can be used to better control the spread of a fungal pathogen at the plot scale? *Ecological Modelling*, **290**, 178–191.

Mavroeidi, V.I., & Shaw, M.W. 2005. Sensitivity distributions and cross-resistance patterns of *Mycosphaerella graminicola* to fluquinconazole, prochloraz and azoxystrobin over a period of 9 years. *Crop Protection*, **24**, 259–266.

McCallum, H., Barlow, N., & Hone, J. 2001. How should pathogen transmission be modelled? *Trends in Ecology & Evolution*, **16**, 295–300.

McDonald, B.A., & Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology*, **40**, 349–379.

McKendry, A.L., Henke, G.E., & Finney, P.L. 1995. Effects of septoria leaf blotch on soft red winter wheat milling and baking quality. *Cereal Chemistry*, **72**, 142–146.

Mikaberidze, A., McDonald, B.A., & Bonhoeffer, S. 2014. Can high risk fungicides be used in mixtures without selecting for fungicide resistance? *Phytopathology*, **104**, 324–331.

Mikaberidze, A., Paveley, N.P., Bonhoeffer, S., & van den Bosch, F. 2017. Emergence of resistance to fungicides: the role of fungicide dose. *Phytopathology*, **107**, 545–560.

Milgroom, M.G. 2015. *Population Biology of Plant Pathogens: Genetics, Ecology and Evolution*. St. Paul: The American Phytopathological Society.

Milgroom, M.G., & Fry, W.E. 1988. A simulation analysis of the epidemiological principles for fungicide resistance management in pathogen populations. *Phytopathology*, **78**, 565–570.

Morton, V., & Staub, T. 2008. A Short History of Fungicides. *APSnet features*.

Nasr, I.N. 2010. Gas chromatographic determination of sulfur residues in tomatoes and squash fruits. *Journal of Applied Sciences Research*, **6**, 1474–1476.

Oliver, R.P. 2016. Fungicide resistance management in practice; mixtures, alternations and cross resistance patterns. *Journal of Plant Pathology*, **98**, S11.

Orr, H.A. 2010a. Fitness and its role in evolutionary genetics. *Nature Reviews Genetics*, **10**, 531–539.

Orr, H.A. 2010b. The population genetics of beneficial mutations. *Philosophical Transactions of the Royal Society of London, B*, **365**, 1195–1201.

Parnell, S, Gilligan, C.A., & van den Bosch, F. 2005. Small-scale fungicide spray heterogeneity and the coexistence of resistant and sensitive pathogen strains. *Phytopathology*, **95**, 632–639.

Parnell, S., van den Bosch, F., & Gilligan, C.A. 2006. Large-scale fungicide spray heterogeneity and the regional spread of resistant pathogen strains. *Phytopathology*, **96**, 549–555.

Parnell, S., Gottwald, T.R., Gilligan, C.A., Cunniffe, N.J., & van den Bosch, F. 2010. The effect of landscape pattern on the optimal eradication zone of an invading epidemic. *Phytopathology*, **100**, 638–644.

Paveley, N., Blake, J., Gladders, P., & Cockerell, V. 2014. *The Wheat Disease Management Guide*. Home-Grown Cereals Authority.

Paveley, N.D., Hims, M.J., Clark, W.S., Bryson, R.J., & Stevens, D.B. 1998. *Appropriate fungicide doses for winter wheat and matching crop management to growth and yield potential*. Tech. rept.

Phillips McDougall. 2013. *R&D Trends for Chemical Crop Protection Products and the Position of the European Market*. Tech. rept.

Pool, R.M., Pearson, R.C., Welser, M.J., Lakso, A.N., & Seem, R.C. 1984. Influence of powdery mildew on yield and growth of Rosette grapevines. *Plant Disease*, **68**, 590–593.

Pringle, A., & Taylor, J.W. 2002. The fitness of filamentous fungi. *Trends in Microbiology*, **10**, 474–481.

Read, A.F., Day, T., & Huijben, S. 2011. The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proceedings of the National Academy of Sciences*, **108**, 10871–10877.

Reuveni, M. 2001. Activity of trifloxystrobin against powdery and downy mildew diseases of grapevines. *Canadian Journal of Plant Pathology*, **23**, 52–59.

Russell, P.E. 2005. A century of fungicide evolution. *The Journal of Agricultural Science*, **143**, 11–25.

Saltelli, A., Ratto, M., Andres, T., Campolongo, F., Cariboni, J., Gatelli, D., Saisana, M., & Tarantola, S. 2004. Introduction to Sensitivity Analysis. *In: Global Sensitivity Analysis. The Primer*.

Sanchez-Vallet, A., McDonald, M.C., Solomon, P.S., & McDonald, B.A. 2015. Is *Zymoseptoria tritici* a hemibiotroph? *Fungal Genetics and Biology*, **79**, 29–32.

Shaw, M.W. 1989a. A model of the evolution of polygenically controlled fungicide resistance. *Plant Pathology*, **38**, 44–55.

Shaw, M.W. 1989b. Independent action of fungicides and its consequences for strategies to retard the evolution of fungicide resistance. *Crop Protection*, **8**, 405–411.

Shaw, M.W. 2000. Models of the effects of dose heterogeneity and escape on selection pressure for pesticide resistance. *Phytopathology*, **90**, 333–339.

Shaw, M.W. 2006. Is there such a thing as a fungicide resistance strategy? A modeller's perspective. *Aspects of Applied Biology*, **78**, 37–44.

Sierotzki, H., & Scalliet, G. 2013. A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. *Phytopathology*, **103**, 880–887.

Skylakakis, G. 1981. Effects of alternating and mixing pesticides on the buildup of fungal resistance. *Phytopathology*, **71**, 1119–1121.

Smith, M. 2014. Risks and hazards to arable farming of the EU pesticide regulations. *Pages 249–252 of: Aspects of Applied Biology 127: Crop Production in Southern Britain: Precision Decisions for Profitable Cropping*.

Staub, T. 1991. Fungicide resistance: practical experience with antiresistance strategies and the role of integrated use. *Annual Review of Phytopathology*, **29**, 421–442.

Staub, T., & Sozzi, D. 1983. Recent practical experiences with fungicide resistance. *Proceedings of the International Congrress of Plant Protection*, 591–598.

Staub, T., Dahmen, H., Urech, P. A., & Schwinn, F. 1979. Failure to select for *in vivo* resistance in *Phytophthora infestans* to acylalanine fungicides. *Plant Disease Reporter*, **64**, 385–389.

Suffert, F., Sache, I., & Lannou, C. 2010. Early stages of septoria tritici blotch epidemics of winter wheat: build-up, overseasoning , and release of primary inoculum. *Plant Pathology*, **60**, 166–177.

te Beest, D.E., Paveley, N.D., Shaw, M.W., & van den Bosch, F. 2013. Accounting for the economic risk caused by variation in disease severity in fungicide dose decisions, exemplified for *Mycosphaerella graminicola* on winter wheat. *Phytopathology*, **103**, 666–672.

Tildesley, M.J., Savill, N.J., Shaw, D.J., Deardon, R., Brooks, S.P., Woolhouse, M.E.J., Grenfell, B.T., & Keeling, M.J. 2006. Optimal reactive vaccination strategies for a foot-and-mouth outbreak in the UK. *Nature*, **440**, 83–86.

Tillman, R.W., Siegel, M.R., & Long, J.W. 1973. Mechanism of action and fate of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) in biological systems: I. reactions with cells and subcellular components of *Saccharomyces pastorianus*. *Pesticide Biochemistry and Physiology*, **3**, 160–167.

van den Berg, F., van den Bosch, F., & Paveley, N.D. 2013. Optimal fungicide application timings for disease control are also an effective anti-resistance strategy: a case study for *Zymoseptoria tritici* (*Mycosphaerella graminicola*) on wheat. *Phytopathology*, **103**, 1209–1219.

van den Berg, F., Paveley, N.D., & van den Bosch, F. 2016. Dose and number of applications that maximise fungicide effective life exemplified by *Zymoseptoria tritici* on wheat - a model analysis. *Plant Pathology*, **65**, 1380–1389.

van den Bosch, F., & Gilligan, C.A. 2008. Models of fungicide resistance dynamics. *Annual Review of Phytopathology*, **46**, 123–147.

van den Bosch, F., Paveley, N., Shaw, M.W., Hobbelen, P., & Oliver, R. 2011. The dose rate debate: does the risk of fungicide resistance increase or decrease with dose? *Plant Pathology*, **60**, 597–606.

van den Bosch, F., Oliver, R., van den Berg, F., & Paveley, N. 2014a. Governing principles can guide fungicide-resistance management tactics. *Annual Review of Phytopathology*, **52**, 175–95.

van den Bosch, F., Paveley, N., van den Berg, F., Hobbelen, P., & Oliver, R.P. 2014b. Mixtures as a fungicide resistance management tactic. *Phytopathology*, **104**, 1264–1273.

van den Bosch, F., Fraaije, B., Oliver, R., van den Berg, F., & Paveley, N. 2015. The Use of Mathematical Models to Guide Fungicide Resistance Management Decisions. *Chap. 4, pages 49–62 of:* Ishii, H., & Hollomon, D.W. (eds), *Fungicide Resistance in Plant Pathogens*, 1 edn. Springer Japan.

van der Plank, J.E. 1963. *Plant Diseases: Epidemics and Control*. New York: Academic Press.

Waggoner, P.E., & Berger, R.D. 1987. Defoliation, disease, and growth. *Phytopathology*, **77**, 393–398.

Walker, A.-S., Ravigne, V., Rieux, A., Ali, S., Carpentier, F., & Fournier, E. 2017. Fungal adaptation to contemporary fungicide applications: the case of *Botrytis cinerea* populations from Champagne vineyards (France). *Molecular Ecology*, **26**, 1919–1935.

Woolfit, M. 2009. Effective population size and the rate and pattern of nucleotide substitutions. *Biology Letters*, **5**, 417–420.

Wu, B., Gokhale, C.S., van Veelen, M., Wang, L., & Traulsen, A. 2013. Interpretations arising from Wrightian and Malthusian fitness under strong frequency dependent selection. *Ecology and Evolution*, **3**, 1276–1280.

Zlof, V., & Sunley, R. 2011. Report of the EPPO workshop on azole fungicides and Septoria leaf blotch control. *EPPO Bulletin*, **41**, 145–148.

Appendices

A

Parameter Values

A.1 Septoria leaf blotch of UK winter wheat

A.1.1 FiveLeaf model

Table A.1: The default values used for each of the parameters used in the FiveLeaf model of septoria leaf blotch on winter wheat.

Symbol	Parameter name	Value	Units	Source
β	Infection rate parameter	1.56×10^{-2}	degree-days^{-1}	See section 2.4.2
$1/\gamma$	Latent period	266	degree-days	Hobbelen *et al.* (2013)
$1/\mu$	Infectious period	456	degree-days	Hobbelen *et al.* (2013)
	High-risk fungicide name	Pyraclostrobin		Hobbelen *et al.* (2011a)
	High-risk fungicide activity	Protectant + eradicant		Hobbelen *et al.* (2011a)
ω_H	High-risk fungicide maximum effect	1		Hobbelen *et al.* (2011a)
θ_H	High-risk fungicide curvature parameter	9.6		Hobbelen *et al.* (2011a)
δ_H	High-risk fungicide decay parameter	1.11×10^{-2}	degree-days^{-1}	Hobbelen *et al.* (2011a)

	Low-risk fungicide name	Chlorothalonil		Hobbelen *et al.* (2011a)
ω_L	Low-risk fungicide maximum effect	0.48		Hobbelen *et al.* (2011a)
θ_L	Low-risk fungicide curvature parameter	9.9		Hobbelen *et al.* (2011a)
δ_L	Low-risk fungicide decay parameter	6.91×10^{-3}	degree-days^{-1}	Hobbelen *et al.* (2011a)
ϕ	Initial resistance frequency	10^{-10}		Assumed
ψ	Initial inoculum density	1.09×10^{-2}		Hobbelen *et al.* (2011a)
$S(T_{\text{GS32}})$	Initial LAI of susceptible leaf	0.05		Hobbelen *et al.* (2011a)
$E_S(T_{\text{GS32}})$	Initial LAI of leaf with latent infection by susceptible strain	0		Hobbelen *et al.* (2011a)
$E_R(T_{\text{GS32}})$	Initial LAI of leaf with latent infection by resistant strain	0		Hobbelen *et al.* (2011a)
$I_S(T_{\text{GS32}})$	Initial LAI of leaf infected by susceptible strain	0		Hobbelen *et al.* (2011a)
$I_R(T_{\text{GS32}})$	Initial LAI of leaf infected by resistant strain	0		Hobbelen *et al.* (2011a)

$R(T_{GS32})$	Initial LAI of dead leaf	0		Hobbelen *et al.* (2011a)
r	Host growth rate parameter	1.26×10^{-2}		Hobbelen *et al.* (2011a)
k	Host carrying capacity	4.2		van den Berg *et al.* (2013)
ν	Primary inoculum decay rate	8.5×10^{-3}	degree-days^{-1}	Hobbelen *et al.* (2011a)
	Spray times	GS32, GS39		Hobbelen *et al.* (2011a)
T_{EMERGE}	Time of emergence of leaf 5	1212	degree-days	van den Berg *et al.* (2013)
T_{GS32}	Time of GS32	1456	degree-days	van den Berg *et al.* (2013)
T_{GS39}	Time of GS39	1700	degree-days	van den Berg *et al.* (2013)
T_{GS61}	Time of GS61	2066	degree-days	van den Berg *et al.* (2013)
T_{GS87}	Time of GS87	2900	degree-days	van den Berg *et al.* (2013)

A.1.2 FiveLeaf sub-models

Table A.2: The value for the infection rate parameter used in each of the FiveLeaf sub-models. The presence of a check mark in a column indicates that the sub-model includes that feature, and the absence that it does not.

Host-limited infection	**Latent infection**	**Fungicide decay**	**Phenology**	**Infection rate parameter (degree-days^{-1})**
✔	✔	✔	✔	0.0156
✔	✔	✔		0.0119
✔	✔		✔	0.0135

✔	✔			0.0103
✔		✔	✔	0.00688
✔		✔		0.00535
✔			✔	0.00625
✔				0.00484
	✔	✔	✔	0.0127
	✔	✔		0.0109
	✔		✔	0.0115
	✔			0.00972
		✔	✔	0.00548
		✔		0.00497
			✔	0.0052
				0.00464

A.1.3 ElevenLeaf model

Main parameters

Table A.3: The default values used for each of the parameter used in the ElevenLeaf model of septoria leaf blotch on winter wheat. The parameters presented in this table are those which are identical across all leaf layers.

Symbol	Parameter name	Value	Units	Source
β_P	Ascospore infection rate parameter	4×10^{-10}	degree-days^{-1}	Kitchen *et al.* (2016)
β_I	Conidiospore infection rate parameter	7×10^{-3}	degree-days^{-1}	Kitchen *et al.* (2016)
b_{DOWN}	Conidiospore downward infection scaling	0.01		Kitchen *et al.* (2016)

b_{UP}	Conidiospore upward infection scaling	0.1		Kitchen *et al.* (2016)
η	Ascospore influx scale parameter	1	degree-days^{-1}	Kitchen *et al.* (2016)
λ	Ascospore influx time parameter	3.5×10^{-3}	degree-days^{-1}	Kitchen *et al.* (2016)
$1/\gamma$	Latent period	250	degree-days	Kitchen *et al.* (2016)
$1/\mu$	Infectious period	500	degree-days	Kitchen *et al.* (2016)
	High-risk fungicide name	Pyraclostrobin		Hobbelen *et al.* (2011a)
	High-risk fungicide activity	Protectant + eradicant		Hobbelen *et al.* (2011a)
ω_H	High-risk fungicide maximum effect	1		See section 2.4.3
θ_H	High-risk fungicide curvature parameter	0.303		See section 2.4.3
δ_H	High-risk fungicide decay parameter	1.11×10^{-2}	degree-days^{-1}	Hobbelen *et al.* (2011a)
	Low-risk fungicide name	Chlorothalonil		Hobbelen *et al.* (2011a)
ω_L	Low-risk fungicide maximum effect	0.606		See section 2.4.3

θ_L	Low-risk fungicide curvature parameter	0.598		See section 2.4.3
δ_L	Low-risk fungicide decay parameter	6.91×10^{-3}	degree-days^{-1}	Hobbelen *et al.* (2011a)
ϕ	Initial resistance frequency	10^{-10}		Assumed
r	Host growth rate parameter	0.034	degree-days^{-1}	Kitchen *et al.* (2016)
τ	Leaf angle projection factor	0.75		See section 2.4.3
q	Leaf thickness	10^{-3}	m	Kitchen *et al.* (2016)

Leaf-specific parameters

Table A.4: The default values used for each of the parameter used in the ElevenLeaf model of septoria leaf blotch on winter wheat. The parameters presented in this table are those which are specific to particular leaf layers. The shaded cells represent that only leaves 1 - 4 extend with time. All values are from Kitchen *et al.* (2016).

Leaf number	**Maximum LAI**	**Emergence time (degree-days)**	**Extension initiation time (degree-days)**	**Senescence initiation time (degree-days)**	**Death time (degree-days)**
1	0.95	1635	1700	2725	2928
2	1.05	1513	1578	2676	2900
3	0.86	1391	1456	2408	2590
4	0.76	1269	1334	2212	2373
5	0.59	1147		1968	2094
6	0.43	1025		1724	1815
7	0.26	903		1480	1536

8	0.26	781		1358	1414
9	0.26	659		1236	1292
10	0.23	537		1090	1139
11	0.23	415		968	1017

A.2 Powdery mildew on grapevine

Table A.5: The default values used for each of the parameter used in the model of powdery mildew on grapevine. In split columns the first value is used before shoot topping and the second after.

Symbol	**Parameter name**	**Value**		**Units**	**Source**
β	Infection rate parameter	1.605	1.688	$cm^{-2}\,d^{-1}$	Burie *et al.* (2011)
$1/\gamma$	Latent period	10		d	Burie *et al.* (2011)
$1/\mu$	Infectious period	10		d	Burie *et al.* (2011)
	High-risk fungicide name	Trifloxystrobin			
	High-risk fungicide activity	Protectant + eradicant			
ω_H	High-risk fungicide maximum effect	1			Assumed
θ_H	High-risk fungicide curvature parameter	4.88			See section 2.5.3

δ_H	High-risk fungicide decay parameter	0.231	d^{-1}	See section 2.5.3
	Low-risk fungicide name	Sulphur		
ω_L	Low-risk fungicide maximum effect	1		Assumed
θ_L	Low-risk fungicide curvature parameter	1.02		See section 2.5.3
δ_L	Low-risk fungicide decay parameter	0.173	d^{-1}	See section 2.5.3
ϕ	Initial resistance frequency	10^{-10}		Assumed
ψ	Initial inoculum density	0.173		Burie *et al.* (2011)
$S(T_{BUD})$	Initial area of susceptible leaf	42.34	cm^2	Burie *et al.* (2011)
$E_S(T_{BUD})$	Initial area of leaf with latent infection by susceptible strain	$(1\text{-}\phi)\psi$		

$E_R(T_{BUD})$	Initial area of leaf with latent infection by resistant strain	$\phi\psi$			
$I_S(T_{BUD})$	Initial area of leaf infected by susceptible strain	0			Burie *et al.* (2011)
$I_R(T_{BUD})$	Initial area of leaf infected by resistant strain	0			Burie *et al.* (2011)
$R(T_{BUD})$	Initial area of dead leaf	0			Burie *et al.* (2011)
$O(T_{BUD})$	Initial area of resistant leaf	0			Burie *et al.* (2011)
r	Host growth rate parameter	0.147	0.032	d^{-1}	Burie *et al.* (2011)
k	Host carrying capacity	26106	2.461×10^8	cm^2	Burie *et al.* (2011)
	Spray times	161, 174		d	Assumed
T_{BUD}	Time of bud break	119		d	Burie *et al.* (2011)
T_{FLO}	Time of flowering	163		d	Mammeri *et al.* (2014)
T_{TOP}	Time of shoot topping	173		d	Mammeri *et al.* (2014)

T_{END}	End of season	245	d	Assumed
	Percentage leaf area lost on shoot topping	20%		Burie *et al.* (2011)